University of Alberta

Community structure of ectomycorrhizal fungi across the subalpine/alpine ecotone of the Canadian Rockies.

by

Gavin W. Kernaghan

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of *Doctor of Philosophy*.

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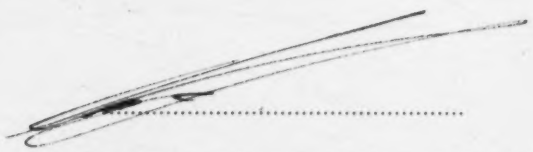

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Abstract

Ectomycorrhizal fungi were analyzed along elevational gradients intersecting tree line in montane Alberta. Sporocarps and ectomycorrhizae were collected in the subalpine forest, the alpine zone and the intervening ecotone on Mt. Rae (Peter Lougheed Provincial Park, 114° 59'W, 50° 36'N) and Mt. Tripoli (Nikanassin Range, 117° 17'W, 52° 52'N). Eighty one species, 22 of which are new records for Canada, were identified from sporocarps. Twenty six were collected as mycorrhizae, and identified by anatomy and comparison of polymerase chain reaction (PCR) amplified DNA with that of sporocarps. Comparisons were made either directly, or by phenetic clustering analyses. Mycorrhizae are described in sufficient detail for re-identification on the basis of anatomy alone.

Soil temperature, moisture, and pH as well as depth of organic material, stand age and proportion of ectotrophic host plants were measured in each habitat and canonical correspondence analysis (CCA) used to determine relationships between these factors and fungal species composition. Diversity of ectomycorrhizal fungi decreased with elevation, in spite of the fact that host plant diversity was highest at the ecotone. Also, a large proportion of the fungi associated with alpine vegetation are non-host specific and can be found in both the alpine zone and the subalpine forest. The observed changes in fungal species diversity and composition were related more closely to host plants than by abiotic factors.

The abundance of non-host specific fungi in the alpine zone is expected to provide a favorable environment for the establishment of conifers above the present tree line. The composition of ectomycorrhizal fungi in the alpine zone should facilitate the upward migration of coniferous forests predicted with global climate change.

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Chapter One

General Introduction

The majority of land plants are dependant on mutualistic symbioses with a wide range of fungi for the improvement of root function. In many woody plants these interactions result in the formation of ectomycorrhizae, in which feeder roots become colonized by fungal hyphae which both envelope root cortical cells and emanate out into the soil. The fungal hyphae then act as extensions of the root system, and in exchange for photosynthates, increase the plant's water (Duddridge *et al.* 1980) and nutrient (Harley and McCready 1950, Melin and Nilsson 1952) uptake capacities and disease resistance (Sinclair *et al.* 1982, Duchesne *et al.* 1987). These mutualisms afford greater plant growth under most conditions and become critical for establishment and support under stressful conditions of montane ecosystems, where soils are poorly developed and climate is extreme (Haselwandter 1987, Väre et al. 1997).

In the Canadian Rocky Mountains, *Abies lasiocarpa* and *Picea engelmannii* co-dominate the subalpine forest and species of *Salix* and *Dryas* are the dominant ectotrophic plants above tree line. Each of these plant genera is associated with a distinct assemblage of ectomycorrhizal fungi (Molina et al. 1992), resulting in a rich ectomycorrhizal mycoflora complete with the characteristic species observed in montane habitats. In spite of this richness, and the special dependance of montane plants on mycorrhizal fungi, the ectomycorrhizal fungi of subalpine and alpine regions remain poorly understood. Several important surveys of sporocarps from European subalpine and alpine zones have been made (Favre 1955, 1960, Kühner and Lamoure 1986, Graf 1994), but North American

records are still poor. With respect to the subterranean environment, the species composition and relative abundance of fungi forming ectomycorrhizae in the alpine zone are essentially unknown (Gardes and Dahlberg 1996). Only recently have efforts been made to identify and describe ectomycorrhizae from European alpine and subalpine zones (Treu 1990, Graf 1996).

A thorough analysis of ectomycorrhizal community structure requires analysis of both sporocarps and ectomycorrhizae. Data on sporocarp occurrence can give the best estimate of species richness, provided fruiting is abundant, as large numbers of taxa can be observed quickly over wide areas. Sporocarp collections are also important for the identification of the associated mycorrhizae. Fructification is often a poor indicator of the actual abundance of ectomycorrhizal fungi in the soil, however, and may not be correlated with the actual abundance of fungi in the soil (Gardes and Bruns 1996). Conversely, ectomycorrhizae are much more difficult to observe, but less ephemeral, more quantifyable and more representative of importance in ecosystem functioning. A major advantage of using data on ectomycorrhizae over that on sporocarps is that proportional abundances can be used to calculate and compare diversity indices among habitats.

Traditionally, fungi colonizing root tips have been identified by *in vitro* re-synthesis (Fortin *et al.* 1980), comparison with published anatomical descriptions (Goodman *et al.* 1997), comparison of cultures obtained from sporocarps and mycorrhizae (Hutchison 1991) or by demonstration of continuity between mantle tissue and sporocarps (Agerer 1991). Comparison of restriction fragment length polymorphism (RFLP) profiles between polymerase chain reaction (PCR) amplified rDNA from mycorrhizae and sporocarps

(Gardes and Bruns 1993) is faster and more reliable than the above methods. The speed, reproducibility and potential for comparison of mycorrhizae with sporocarps collected at different times and locations has made DNA matching the method of choice for the species level identification of ectomycorrhizae.

Anatomical characterization is also necessary for the identification of ectomycorrhizae. Comparison of fungal tissue between mycorrhizae, associated sporocarps and literature descriptions gives the first line of evidence as to the possible identity of the mycobiont. Putative identifications can then be confirmed by comparing mycorrhizal rDNA with that extracted from select sporocarps from the same site. Detailed anatomical characterization is also critical for subsequent re-identification based on anatomy alone.

In the Front Range of the Canadian Rockies, the mature subalpine forest reaches its upper elevational limit at approximately 2,000 m, beyond which low growing alpine shrubs begin to dominate. At the zone of transition between these habitats, conifers form discrete, stunted, multi-stemmed islands or krummholz (Wardle 1968) resulting in a mosaic of alpine and subalpine vegetation. Ecotones tend to harbour relatively diverse communities (Hansen *et al.* 1992, Risser 1995) due to the unique combination of components from the adjacent habitats. This is the "edge effect" described by Leopold (1933), which has been shown to occur in a wide range of ecosystems (Hansen *et al.* 1992). Because many of the plant genera at the subalpine/alpine ecotone in the Canadian Rockies are obligately ectomycorrhizal, we might expect distributional patterns of the ectotrophic vegetation, such as increased diversity at the ecotone, to be reflected in mycorrhizal fungi.

We might also expect ectomycorrhizal fungi to increase the elevational range of the subalpine forest, as mycorrhizal colonization better enables host plants to tolerate harsh conditions (Moser 1967). The elevational position of montane ecotones (tree line) may then be determined not only by climatic and soil conditions (Hansen-Bristow *et al.* 1988), but also by the structure of the ectomycorrhizal community .

Objectives

The objectives of this study are: 1) to describe the species composition of ectomycorrhizal fungi at and near tree line at two sites in the Front Range of the Canadian Rockies, based on collections of sporocarps and ectomycorrhizae, 2) to develop PCR-based methods to be used in combination with more traditional anatomical characterizations to identify and describe the components of these communities and 3), to compare species composition, richness and diversity of ectomycorrhizal fungi among the subalpine forest, the alpine zone and the intervening transition zone and relate these to distributional patterns in ectotrophic vegetation.

In Chapter Two the ectomycorrhizal communities of the two sites are characterized on the basis of sporocarp collections. In Chapter Three, the Russulaceae is used as a model for the development of methods for the characterization and identification of ectomycorrhizae. Chapter Four makes use of these methods to identify and describe a wider variety of ectomycorrhizae from the sites. Phenetic clustering of RFLP data for genus level identification is also introduced. Chapter Five compares the richness and diversity of ectomycorrhizal fungi across the ecotone and uses canonical correspondence analysis to further describe the distribution of mycorrhizal fungi on the sites.

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Chapter Two

Ectomycorrhizal fungi at tree line in the Canadian Rockies[1]

Introduction

Montane ecosystems are often rich in ectomycorrhizal fungi and include fungal species endemic to high elevation environments. European records of montane ectomycorrhizal fungi include those from *Picea*-dominated subalpine forests (Favre 1960, Treu 1990, Rücker *et al.* 1990, Bieri *et al.* 1992) and the well documented *Salix*-dominated alpine habitats (Favre 1955, Lamoure 1977, 1978, 1987, Horak 1960, 1987, Kühner and Lamoure 1986, Senn-Irlet 1988, 1993, Bon 1992, Graf 1994). In North America, montane ectomycorrhizal fungi are less known, but the number of species collected with subalpine conifers (Overholts 1919, Kauffman 1921, Cázares 1992, Moser *et al.* 1995a, Moser and Ammirati 1996) and alpine *Salix* (Moser and McKnight 1987, Moser 1993) is increasing.

At higher elevations in the Front Range of the Canadian Rockies, the mature, subalpine forests of *Picea engelmannii* and *Abies lasiocarpa* begin to give way to the low growing shrubs which dominate the alpine vegetation. At the ecotone, or zone of transition between these habitats, conifers form discrete, stunted, multi-stemmed islands or krummholz (Wardle 1968) resulting in a mosaic of alpine and subalpine vegetation.

Ecotones are potentially species rich, because of the unique combination of components from adjacent habitats (Risser 1995). Although the mycota of subalpine/alpine

[1]A version of this manuscript has been accepted for publication in Mycotaxon **69**: 39-80, 1998.

ecotones has received some attention in Europe (Moser 1967a, Gulden and Lange 1971, Moser 1982, Jacobsson 1984), the composition of ectomycorrhizal fungi at tree line in North America is essentially unknown.

This paper presents a list of ectomycorrhizal fungi collected at tree line, annotated with information on habitat, host associations and distributions. Taxonomic problems and morphological features are also discussed where pertinent. Many of the species identified represent new records for Alberta or Canada.

Materials and Methods

Sporocarps were collected between 2,000 and 2,200 m asl on the southeast slope of Mt. Tripoli, in the Nikanassin Range, Alberta (117^0 17'W, 52^0 52'N) and between 2,300 and 2,500 m asl on the southwest slope of Mt. Rae, Peter Lougheed Provincial Park (114^0 59'W, 50^0 36'N)(Fig. 2.1). Sites were approx. 10 ha and covered by equal areas of the three vegetation types: (1) subalpine forest, co-dominated by *Picea engelmannii* Parry and *Abies lasiocarpa* (Hook.) Nutt. (both at least 100 years old, based on increment cores), with closed canopies and sparse understory vegetation, (2) krummholz (stunted) *Picea* and *Abies*, forming islands separated by areas dominated by ericaceous species and the ectomycorrhizal species, *Salix barrattiana* Hook., *S. glauca* L., *S. arctica* Pallas, *Dryas octopetala* L., *D. integrifolia* M.Vahl, *Polygonum viviparum* L. and *Kobresia myosuroides* (Vill.) Fiori and Paol., and (3) alpine vegetation, similar to krummholz zone but without conifers. Mt. Tripoli, located 350 km northwest of Mt. Rae, receives more precipitation (see below) and has lower temperatures, and, as a result, has a lower (300 m)

tree line. Other differences include the occurrence of *Betula glandulosum* Michx. between krummholz conifers on Mt. Tripoli and scattered *Larix lyallii* Parl. in the subalpine forest and krummholz zone on Mt. Rae.

Soils are Dystric and Eutric Brunisols and Orthic and Orthic Humic Regosols (Trottier 1972, Mortimer 1978). Average monthly snowfall reaches 28 cm in June and 47 cm in September. During July and August the ground is relatively snow-free, and mean daily temperatures range from 6 to 10^0 C. Mean monthly precipitation during July and August averages 97 mm at Mt. Tripoli and 72 mm at Mt. Rae (Environment Canada, Archives of Climatological Data).

Sites were visited monthly between June and Sept. 1994-97. Sporocarps of all fungi in putatively ectomycorrhizal genera (based on Molina et al. 1992) were collected. Corticioid fungi were found by rolling logs, and sporocarps of the hypogeous taxa collected were partially erumpent. Representative collections are deposited in the University of Alberta Cryptogamic Herbarium (ALTA). Microscopic examinations were made under high magnification in H_20, Melzer's reagent or sulphovanillin where appropriate. To determine spore size, 20-30 mature spores were measured in H_2O under oil.

Only published accounts that include habitat (host) data or important information on distributions are included in annotations. Herbarium records and foray lists are not included. Salient characters are described for taxa when discrepancies exist in the literature. Determination of new records for Alberta and western Canada was based (in part) on databases of Canadian macrofungi compiled by S.A. Redhead (Redhead 1997,

Redhead unpublished data) and on Ginns and Lefebvre (1993).

Results

A total of 81 species in 29 genera and 13 families were documented. Twenty two species are new records for Canada, 35 to western Canada, and 40 to Alberta. Two genera are new records for Alberta. Species richness decreased with elevation: 65 species in 28 genera were collected in the subalpine forest, 41 species in 17 genera in the krummholz zone and 14 species in seven genera in the alpine zone. Taxa identified from the krummholz zone fell into three major assemblages with respect to host associations (Table 2.1): (1) 20 conifer associates, many with circumboreal distributions, likely to form ectomycorrhizae with krummholz *Abies*, *Picea* and (or) *Larix*, (2) 7 angiosperm associates, many with circumpolar and alpine distributions, likely to form ectomycorrhizae with dwarf *Salix*, *Betula* or *Dryas* spp. and (3) 14 species with little host specificity which are likely to form mycorrhizae with both the angiosperms and gymnosperms present at tree line.

Species collected

Ascomycota

Cenococcum geophilum Fr.: Fr., Syst. Mycol. III: 66. 1830.

Common throughout the northern hemisphere. Mycorrhizal with all Pinaceae, Betulaceae, Fagaceae and Salicaceae, and some species of Rosaceae, Myrtaceae and Tiliaceae (Trappe 1962, 1964). Distinctive sclerotia and ectomycorrhizae are formed, but

its precise position within the Ascomycota is unknown (Lobuglio *et al.* 1996). Collected repeatedly at both sites and in all habitats.

Material examined: Mt. Rae: subalpine forest 07 21 94 (ALTA 10361).

Hydnotrya cubispora (Bessey and B.E. Thompson) Gilkey, Oregon State Monogr., Stud. Bot. 1: 23. 1939.

A widely distributed but uncommon North American conifer associate. Hypogeous under *Picea, Pinus, Pseudotsuga* and (or) *Tsuga* in northern and northwestern North America (Abbott and Currah 1997). Under *Tsuga, Abies, Betula* and *Acer* in Maine and Michigan (Bessey and Thompson 1920) and in the southeastern U.S. under *Tsuga* (Miller and Miller 1982). Also with introduced *Picea sitchensis* in the U.K. (Pegler *et al.* 1993). The genus *Hydnotrya* has not been reported previously from Alberta.

Material examined: Mt. Tripoli: 09 07 97 (ALTA 10373).

Basidiomycota

Amanitaceae

Amanita vaginata (Bull.:Fr.) Vitt., Tent. Mycol. Amanitarum Illustr. p. 30. 1826. *sensu lato*

A widespread and variable angiosperm associate. Common and widely distributed in North America and Europe in mixed coniferous and deciduous forests (Jenkins 1986), also well known from arctic (Lange 1955, Miller 1982, Petersen 1977) and alpine habitats (Favre 1955, Kühner and Lamoure 1986, Bas 1982), where it is associated with *Salix* and

Betula. Many forms and varieties are recognized (see Bas 1977), some of which are found with *Betula* near tree line (Lange and Skifte 1967, Gulden and Lange 1971, Jacobsson 1984, Watling 1987). Our material, collected near *Betula glandulosum*, *Salix barrattiana* and *Dryas octopetala*, has a fawn-coloured pileus drying to pale grey, up to 6 cm in diam., a white stipe up to 10 cm long and globose to subglobose spores, 8.7-12.0 x 8.7-12.6 μm, **Q** = 1.06.

Material examined: Mt. Tripoli: krummholz zone 08 05 94 (ALTA 10359); 08 30 97 (ALTA 10360). Devonian Botanic Garden, AB: S. Abbott 07 26 88 (UAMH 8576).

Boletaceae

Rhizopogon rubescens (Tul.) Tulasne, Giorn., Bot. Ital. 2: 58. 1844.

A common and widespread hypogeous conifer associate. Widely distributed in North America, usually the most common species of the genus in the north (Harrison and Smith 1968), also in Europe. Associated with Pinaceae, especially 2- and 3-needle pines (Miller 1986), but also with *Abies, Picea, Pseudotsuga, Tsuga* and *Quercus* (Molina and Trappe 1994). Ectomycorrhizae synthesized *in vitro* on *Pinus radiata, Pinus sylvestris* (Molina and Trappe 1994) and *Larix laricina* (Sampson and Fortin 1988). Although the spores of our material (7.0 - 9.9 x 3.0 - 3.7 (4.2)μm) are slightly smaller than those described by most authors, Smith and Zeller (1966) noted similar sized spores in some of their western collections.

Material examined: Mt. Rae: subalpine forest 07 21 94 (ALTA 10362); 07 25 95 (ALTA 10363); 09 29 95 (ALTA 10364); 08 16 97 (ALTA 10365). Smith Dorrian

Valley, AB: 08 17 97 (ALTA 10366).

Suillus aeruginascens (Fr.) Snell, Lloydia 7: 25. 1944.

Circumboreal with *Larix* spp. Common in northern North America; Idaho, Michigan, New York, Oregon, and Québec with *L. laricina* and *L. occidentalis* (Pomerleau and Smith 1962), as well as Finland with *L. sibirica* (Heikkilä 1982) and the Austrian Alps with *L. decidua* (Moser 1982). Ectomycorrhizae have been synthesized on *Larix laricina* (Sampson and Fortin 1988) and *L. occidentalis*, *Pseudotsuga menziesii* and *Picea sitchensis* (Molina and Trappe 1982). Synonymous with *Fuscoboletinus aeruginascens* (see Kretzer *et al.* 1996). This is the first report of this species with *Larix lyallii*.

Material examined: Mt. Rae: krummholz zone 08 4 95 (ALTA 10374); subalpine forest 08 04 95; 08 16 97 (ALTA 10375).

Suillus cavipes (Opat.) Smith and Thiers, Contrib. Monogr. North Am. spec. *Suillus* 30. 1964.

Circumboreal, with *Larix* spp. Following the range of *L. laricina* and *L. occidentalis* (Smith and Thiers 1964, Thiers 1975) and with *L. sibirica* in Northern Finland (Heikkilä 1982) and *L. decidua* in the Alps (Moser 1982)(as *Boletinus cavipes*). This is the first report with *L. lyallii*.

Material examined: Mt. Rae: subalpine forest 08 16 97 (ALTA 10377).

Chaetoporellaceae

Amphinema byssoides (Pers.:Fr.) Eriksson, Symb. Bot. Upsal. 16: 112. 1958.

Widely distributed in North America and Europe, fruiting on rotting wood of a variety of angiosperms and gymnosperms (Eriksson and Ryvarden 1973, Ginns and Lefebvre 1993). Reported to form ectomycorrhizae with *Pinus strobus* (Fassi and De Vecchi 1962), *P. banksiana* Lamb. (Danielson 1984), *Picea glauca, P. pungens* (Danielson and Pruden 1989), *P. abies* (Weiss 1991) and *P. sitchensis* (Ingelby 1990). Also with *Picea engelmannii*, *Abies lasiocarpa*, *Dryas octopetala* and *Salix* spp. (Kernaghan and Currah, unpublished data). Much of the material collected during this study is from dead wood and prostrate branches of krummholz *Abies lasiocarpa.* **Material examined**: Mt. Rae: alpine zone 09 19 97 (ALTA 10276); krummholz zone 08 16 95 (ALTA 10350). Mt. Tripoli: krummholz zone 08 05 94 (ALTA 10351); 08 05 94 (ALTA 10352); subalpine forest 08 30 97 (ALTA 10353).

Cortinariaceae

Cortinarius albonigrellus Favre, Rés. rech. sci. entr. Parc Nat. suisse, 5: 127. 1955. (subgenus *Telamonia*)

An arcto-alpine *Salix* associate. With dwarf alpine *Salix* in the Alps (Favre 1955, Lamoure 1977, Kühner and Lamoure 1986), Wyoming (Moser and McKnight 1987, Moser 1993), and the Greater Yellowstone Area (Moser *et al.* 1995b). Our material agrees with Horak's (1987) description of Favre's original material and with the description of Lamoure (1977). Pileus 1.5-2.0 cm, dark grey-black, glabrous. Stipe with

persistent, peronate white veil; young lamellae and context pale yellow brown. Spores variable in size, 7.7-9.5 (11.0) x 4.0-5.6 μm, subcylindric to ellipsoid, finely verrucose, not all subcylindric as described by Horak (1987). Moser and McKnight (1987) describe *C. albonigrellus* from Wyoming with spores 8.8-12.3 x 5.3-7.0μm. Not previously reported from Canada.

Material examined: Mt. Rae: alpine zone 08 16 95 (ALTA 10138); krummholz zone 08 16 95 (ALTA 10139); 09 29 95 (ALTA 10140).

Cortinarius brunneus Fr., Epicr. Syst. Mycol., p. 298. 1838. (subgenus *Telamonia*)

With northern and coastal conifers and (rarely) with alpine vegetation. Known from central and northern Europe, in mixed boreal and montane coniferous forests, usually in oligotrophic *Picea* forests (Brandrud *et al.* 1992, Gulden *et al.* 1992, Arnold 1993). Also with subalpine *Betula* and *Salix* in northern Norway (Lange and Skifte 1967), *Pinus sylvestris* in northern Finland (Väre *et al.* 1996) and in the Austrian Alps up to timberline (Moser 1982). In North America, more common under conifers in the Pacific Northwest than elsewhere (Smith *et al.* 1979). In Washington, under second growth *Pseudotsuga menziesii* (Smith 1944) and in arctic tundra, northern Québec (Hutchison *et al.* 1988). Not previously reported from western Canada.

Material examined: Mt. Rae: subalpine forest 07 25 95 (ALTA 10145). Mt. Tripoli: subalpine forest 07 08 94 (ALTA 10146); 07 18 95 (ALTA 10147). Wildhay River AB: 08 06 95 (ALTA 10148).

Cortinarius calochrous (Pers.: Fr.) Fr., Epicr. Syst. Mycol., p. 267. 1838. (subgenus *Phlegmacium*).

A circumboreal conifer associate. Known from European boreal and montane forests with *Abies*, *Picea* and (or) *Pinus* (Moser 1960, Brandrud *et al.*1990, 1994). In North America, collections of *C. calochrous* in coniferous forests from Washington (Smith 1944, Stuntz 1981) are likely subsp. *coniferarum*. Also in the Greater Yellowstone Area (Moser *et al.* 1995b)(as *C. calochrous fo. caroli* Vel.) and the Canadian Rockies (Schalkwijk-Barendsen 1991). Mycorrhizae are described on *Picea engelmannii* (Chapter Four of this thesis).

C. calochrous s.l. forms one of the most difficult taxonomic complexes in the subgenus *Phlegmacium* (Brandrud *et al.* 1990). Moser (1960) recognized var. *calochrous* from deciduous forests, var. *coniferarum* Mos. (with larger spores) from coniferous forests and *fo. caroli* (also with larger spores and with violaceus stipe pigments), mainly from deciduous forests. Brandrud *et al.* (1992) elevated these varieties to subspecies and described subsp. *calochrous* from deciduous forests, subsp. *coniferarum* (Moser) Brandrud (with larger spores) from conifer forests, and varieties of each. My material has consistently violaceus stipe apices, spores measuring 9.8-11.5 (13) x (5.6) 6-7 μm and seems closest to subsp. *coniferarum* (Mos.) Brandrud.

Material examined: Mt. Rae: subalpine forest 08 16 95 (ALTA 10185). Mt. Tripoli: subalpine forest 08 04 95 (ALTA 10186); 08 15 95 (ALTA 10187).

Cortinarius chrysomallus Lamoure, Trav. Sci. Parc Nat. Vanoise 8: 135. 1977.

(subgenus *Telamonia*)

An alpine *Salix* associate, also with subalpine *Picea*. Collected with dwarf alpine *Salix* in the Alps (Lamoure 1977, Kühner and Lamoure 1986, Senn-Irlet 1993, Bon 1992, Graf 1994), Sweden and Norway (Lamoure 1977). In Wyoming, Montana, California and Washington with shrubby and dwarf *Salix* and *Picea engelmannii* (Moser and McKnight 1987, Moser 1993, Moser *et al.* 1995b). Our material agrees well with the description of Lamoure (1977). Pileus up to 3.5 cm, red brown, glabrous. Stipe dingy greyish, booted with bright yellow universal veil remnants, which turn red in 5% KOH. Young lamellae pale grey-brown with mauve tones. Context with distinct mauve tint. Spores (7.4-8.8 x 4.1-5.6 μm) are slightly smaller than reported by Lamoure (1977), but those depicted by Moser and McKnight (1987) show similar variation among collections. *Cortinarius chrysomallus* appears to be a predominantly alpine species but it has been collected at lower elevations (Lamoure 1977) and near *Picea* (Moser and McKnight 1987). Our material was collected under *Picea engelmannii* and *Abies lasiocarpa*. Not previously reported from Canada.

Material examined: Mt.Tripoli: subalpine forest 08 04 95 (ALTA 10234). Jasper National Park: 07 15 95 (ALTA 10149).

Cortinarius clandestinus Kauffman, North American Flora 10: 324. 1932 (subgenus *Leprocybe*)

A North American conifer associate. Associated with *Tsuga* and *Pseudotsuga* in Washington (Kauffman 1932, Stuntz 1981), also in Colorado, Idaho, Oregon, Michigan

and New York (Kauffman 1925, Kauffman and Smith 1933). *C. cotoneus* is well known from European deciduous forests (Høiland 1980, Brandrud *et al.* 1992) and is morphologically similar to *C. clandestinus* (Stuntz 1981, Arora 1986). Reports of *C. cotoneus* from coniferous forests in North America (e.g. Smith 1939, 1944, Phillips 1991) may be based on collections of *C. clandestinus*. Not previously reported from Canada.

Material examined: Mt. Rae: krummholz zone 08 16 97 (ALTA 10215).

Cortinarius colus Fr., Monographia, p. 102. 1851. (subgenus *Telamonia*)

A circumboreal *Picea* associate, also with *Pinus*. Associated with *Picea excelsa* in central Europe and Scandinavia (Moser 1965). Also in northern Finland, with *Pinus sylvestris* (Väre *et al.* 1996), and Germany, with conifers, especially *Picea* (Arnold 1993). Also collected in Washington (Smith 1944). Smith (1939) tentatively identified a *Telamonia* from northern California under *Abies* as *C. colus* (violet stipe apex and purple-red or vinaceous universal veil and spores 6-7 X 4-5 μm). My material has a red brown, hygrophanous pileus, 2-4 cm in diam., pale grey-brown lamellae, greyish white stipe, 3-5 x 0.7-1.5 cm, white cortina and bright orange universal veil (purple in 5% KOH) forming persistent boot at stipe base. Spores (7.4) 7.8-9.0 x 4.7-5.8 μm. Not previously reported from Canada.

Material examined: Mt. Rae: subalpine forest 08 16 95 (ALTA 10150); 08 16 97 (ALTA 10151). Mt. Tripoli: subalpine forest 08 15 95 (ALTA 10152).

Cortinarius crassus Fr., Epicr. Syst. Mycol., p. 257. 1831. *sensu* Smith. (subgenus *Phlegmacium*)

A montane conifer associate. Well known from European conifer forests up to montane elevations, especially with *Picea* (Moser 1960, Henry 1984, Brandrud *et al.* 1992, Gulden *et al.* 1992), but also with *Pinus* and *Betula* in Finnish Lapland (Kallio and Kankainen 1964). In North America it is found in northern conifer forests and not uncommon in the western mountains, especially at higher elevations (Smith 1939, 1944, Smith *et al.* 1979, Stuntz 1981, Arora 1986), but differs from European material in lacking filiform cheilocystidia (see Moser 1960). Not previously reported from Canada. **Material examined**: Mt. Tripoli: subalpine forest 09 11 94 (ALTA 10179); 09 07 97 (ALTA 10180).

Cortinarius delibutus Fr., Epicr. Syst. Mycol., p. 276. 1838. (subgenus *Myxacium*)

Associated with angiosperms (especially *Betula*) at higher elevations and latitudes and with conifers elsewhere. In northern Europe, with subalpine *Betula* and *Fagus*, and at higher elevations with dwarf *Salix* and *Betula nana* (Lange and Skifte 1967, Gulden and Lange 1971, Jacobsson 1984, Brandrud *et al.* 1994), also with *Picea* (Gulden *et al.* 1992, Brandrud *et al.* 1994, Bieri 1995) and *Pinus* (Favre 1960). Also known from Britain with *Fagus* and *Betula* (Orton 1955). In North America, it is reported from New York, Nova Scotia and Ontario in mixed woods (Pomerleau 1980) and in the west from Colorado with montane conifers (Kauffman 1921, 1932) and in the Greater Yellowstone Area (Moser *et al.* 1995b). Also with *Salix* and *Betula* in Alaskan tundra (Ammirati and Laursen 1982)

and in Greenland (Lange 1957). Ectomycorrhizae have been synthesized *in vitro* between *C. delibutus* and *Tsuga heterophylla* (Kropp and Trappe 1982).

Cortinarius griseoluridus Kauff. is a similar species, also associated with *Picea* and *Abies* in the Rocky Mountains (Smith *et al.* 1979). It differs from *C. delibutus* in having violaceus tones to the pileus and larger spores (8-10 x 6.5-8 μm) (Smith *et al.* 1979). The spores of our material measure 7.5-8.5 x 6.5-7.5 μm.

Material examined: Mt. Rae: subalpine forest 08 12 94 (ALTA 10199); 08 16 95 (ALTA 10200); 07 25 95 (ALTA 10201); 08 16 97 (ALTA 10202); 09 19 97 (ALTA 10203). Mt.Tripoli: krummholz zone 08 04 95 (ALTA 10204); 08 15 95 (ALTA 10205); subalpine forest 08 4 95 (ALTA 10206); 08 15 95 (ALTA 10207); 09 11 94 (ALTA 10208). Bruderheim, AB: E. Nagasawa 1982 (CFB 21211).

Cortinarius dilutus Fr., Epicr. Syst. Mycol. p. 305. 1838. (subgenus *Telamonia*)

A northern associate of *Picea*. In central Europe and throughout the Alps, primarily with *Picea* but also in mixed woods, especially in swampy areas (Moser 1984, Arnold 1993). Also in Fennoscandia, in coniferous or *Betula* forests (Hansen and Knudsen 1992). With montane conifers, Colorado, Washington, Oregon and Adirondaks (Kauffman 1921, 1932, Smith 1944) and with *Picea engelmannii* in the Greater Yellowstone area (Moser *et al.* 1995b). In Greenland with *Salix* (Lange 1957) (as *C. saturninus*) and Nova Scotia with *Picea* (Gourley 1983) or *Fagus* and *Abies* (Smith and Wehmeyer 1936) and *Picea* in northern Québec (Hutchison *et al.* 1988) (as *C. saturatus*). Synonymous with *C. saturatus* Lge. *fide* Moser (1984). Not previously reported from western Canada.

Material examined: Mt. Tripoli: subalpine forest 08 14 95 (ALTA 10153).

Cortinarius evernius (Fr.:Fr.) Fr., Epicr. Syst. Mycol., p. 294. 1838. (subgenus *Telamonia*)

Circumboreal, generally with *Picea* up to subalpine elevations, often in hygric areas. In central Europe with *Picea* (Gulden *et al.* 1992, Arnold 1993) and in Fennoscandia, with *Picea*, subalpine *Betula* or mixed *Picea* and *Fagus* (Brandrud *et al.* 1990, Kallio and Kankainen 1966, Moser 1967b). In northern U.S. and Canada with conifers in wet or mossy habitats, not common (Kauffman 1932, Smith *et al.* 1979). Reported from Washington with *Abies lasiocarpa, A. amabilis* and *Tsuga heterophylla* (Moser and Ammirati 1996). Not previously reported from western Canada.

Material examined: Mt. Rae: krummholz zone 09 29 95 (ALTA 10154); 09 19 97 (ALTA 10155).

Cortinarius favrei Mos. ex Henderson, Notes Roy. Bot. Gard. Edinb., 22: 593. 1958. (subgenus *Myxacium*)

A circumpolar and alpine *Salix* associate. With dwarf alpine *Salix* spp. in Norway (including Svalbard) (Ohenoja 1971, Gulden *et al.* 1985, Gulden and Lange 1971), the Alps (Favre 1955, Senn-Irlet 1988, 1993, Graf 1994, Kühner and Lamoure 1986), Scotland (Watling 1987) and Greenland (Petersen 1977). Also reported with *Betula* in Swedish alpine heaths (Jacobsson 1984). With dwarf alpine *Salix* in North America; Wyoming (Moser and McKnight 1987, Moser 1993), Alaska (Kobayashi *et al.* 1967,

Ammirati and Laursen 1982), and the Greater Yellowstone Area (Moser *et al.* 1995b). Synonymous with *C. alpinus* Boud. *fide* Ohenoja (1971). Not previously reported from Canada.

Material examined: Mt. Tripoli: alpine zone 09 07 97 (ALTA 10209); krummholz zone 08 15 95 (ALTA 10210); 09 11 95 (ALTA 10211).

Cortinarius fulminoides (Mos.) Mos., in Gams, Kleine Kryptogamenflora, II, b/2, Basidiomyceten II, Ed. 3, p. 284. 1967. (subgenus *Phlegmacium*)

A montane *Picea* associate. Reported from the Austrian Alps with *Picea excelsa* (Moser 1960). Our material fits Moser's (1960) description closely. Pileus fulvus-orange, up to 8 cm in diam., slightly fibrillose towards margin, spotting green, reddening in 5% KOH. Young lamellae pallid grey brown. Cortina white, scant. Stipe white, up to 5 x 2 cm., with a bulbous base. Context thick, yellowish, localized areas becoming green. Spores amygdaliform (7.6) 8.0-9.0 x 5-5.5 (5.9) μm. The context of our material did not become orange-brown in KOH as in Moser's description. A previous Canadian report by Currah *et al.* (1989) is based on misidentified material (UAMH M0457, M0487).

Material examined: Mt. Tripoli: krummholz zone 09 11 94 (ALTA 10181); subalpine forest 09 11 94 (ALTA 10182).

Cortinarius galerinoides Lamoure, Trav. Sci. Parc Nat. Vanoise, 8: 133. 1977. (subgenus *Telamonia*)

A circumpolar and alpine *Salix* associate. Reported from the French Alps (Kühner and Lamoure 1986, Lamoure 1977) with dwarf alpine *Salix* and from Wyoming with

shrubby alpine *Salix* (Moser 1993). Our material fits the description of Moser (1993). Sporocarps resembling *Galerina* in habit, pileus up to 1.2 cm, red brown, hygrophanous, black in 5% KOH. Stipe more or less concolorous with pileus. Lamellae rust brown. Cortina white, very scant. Spores ellipsoid (7.0) 7.6-10.3 x (4.2) 4.6-5.5 (5.9) μm. Not previously reported from Canada.

Material examined: Mt. Tripoli: alpine zone 08 15 95 (ALTA 10156); krummholz zone 08 15 95 (ALTA 10157).

Cortinarius glaucopus Fr., Epicr. Syst. Mycol., p. 264. 1838. (subgenus *Phlegmacium*)

A common conifer associate. Throughout Europe with *Picea* and *Pinus* up to 1900 m (Moser 1960), rarely in deciduous woods (Brandrud *et al.* 1994). In Britain with conifers (Orton 1955) and in the eastern United States with deciduous trees (Kauffman 1932). In western North America it is one of the most common *Cortinarii* along the coast (Smith 1939, Stuntz 1981), often in large troops under *Pseudotsuga menziesii* (Smith 1939) or *Abies* and *Picea* (Smith *et al.* 1979). Also with *Picea* in the Rockies (Arora 1986, Currah *et al.* 1989).

Moser (1960) recognized *C. glaucopus* var. *acyaneum* Mos., which lacks blue pigments, and var. *olivaceum* Mos., with strong green tones towards the centre of the pileus. Smith (1939) reported a green form from California as did Stuntz (1981) from the Pacific Northwest. Our material corresponds to the typical variety, lacking green tones.

Material examined: Mt. Tripoli: 08 30 97 (ALTA 10183); 09 07 97 (ALTA 10184).

Cortinarius hinnuleus (Sowerby: Fr.) Fr., Epicr. Syst. Mycol., p. 296. 1838. var. *favreanus* Bon, Doc. Mycol. 22: 52. 1992. (subgenus *Telamonia*)

Circumpolar and alpine. *Cortinarius hinnuleus s.l.* is common in Europe (Brandrud *et al.* 1990, Arnold 1993) and North America (Kauffman 1932, Pomerleau 1980) with woody angiosperms including *Quercus, Betula* and *Fagus* as well as with conifers. *Cortinarius hinnuleus* var. *favreanus* is a circumpolar and alpine associate of *Salix* and *Dryas*, known from the Swiss and French Alps with dwarf *Salix* or *Dryas* (Favre 1955, Kühner and Lamoure 1986, Lamoure 1977, Senn-Irlet 1988), Greenland with *Salix* (Lange 1957) and Wyoming with alpine *Salix* (Moser 1993). Our material agrees well with the description of Moser (1993). Pileus hygrophanous to yellow-brown, up to 3.5 cm in diam., lamellae subdistant, pale ochre, stipe yellow brown, partial veil whitish to pale ochre, spores 8.3-9.7 (-10.4) x (4.8) 5.2-6.4 μm, smell slightly earthy, gregarious with *Salix* spp. Synonymous with *C. hinnuleus* var. *gracilis* R. Maire *fide* Moser (1993). Not previously reported from Canada.

Material examined: Mt. Rae: krummholz zone 07 25 95 (ALTA 10158); 08 16 95 (ALTA 10159); 07 08 94 (ALTA 10160).

Cortinarius inops Favre, Rés. rech. sci. entr. Parc Nat. suisse 5: 136. 1955. *sensu* Möller. (subgenus *Telamonia*)

Circumpolar and alpine. With dwarf alpine *Salix* or *Dryas*; in the Swiss, French and Italian Alps (Kühner and Lamoure 1986, Lamoure 1978, Favre 1955), Scandinavia (Lamoure 1978, Hansen and Knudsen 1992), Faeröe islands (Möller 1945), Greenland

(Lange 1957), Iceland (Larsen 1932), Wyoming (Moser 1993) and the Greater Yellowstone Area (Moser *et al.* 1995b). Our material agrees well with Möller's (1945) description (as *C. pusillus*). Pileus up to 2 cm in diam., convex with an involute margin and prominent umbo, dark brown, radially fibrillose, hygrophanous, sometimes splitting to reveal pallid context. Lamellae ochre to cinnamon. Stipe up to 2.5 cm long, hollow, ochre-brown and covered with white fibrils, sometimes with a pinkish cast or pinkish basal hyphae. Spores 6.8-9.0 x 5.0- 6.5 μm, broadly elliptic to ovoid, coarsely verrucose. The purple-blue tints described by Möller (1945) were not observed. Although short clavate cheilocystidia are described by Favre (1955), Lamoure (1977) and Moser (1993), the sterile cells along the edge of the lamellae in our material are more or less undifferentiated, as described by Möller (1945). Synonymous with *C. pusillus fide* Moser (1993). Not previously reported in Canada.

Material examined: Mt. Rae: alpine zone 07 25 95 (ALTA 10161). Mt. Tripoli: krummholz zone 07 8 94 (ALTA 10162); 07 18 95 (ALTA 10163).

Cortinarius multiformis (Fr.) Fr., Epicr. Syst. Mycol., p. 263. 1838. var. *coniferarum* (Moser) Nezdoiminogo, Shlyapochnye Griby SSSR Rod Cortinarius Fr.: 39. 1983. (subgenus *Phlegmacium*)

Circumboreal. *C. multiformis s.l.* occurs in Europe throughout the range of *Picea* (Brandrud *et al.* 1990), also with *Betula*, *Salix* and *Pinus* in Finnish Lapland (Kallio and Kankainen 1966) and Greenland with *Salix* (Lange 1957). Also common and widely distributed under conifers, in the Rocky Mountains, northern U.S. and Canada (Kauffman

1932, Smith *et al.* 1979); under *Abies* in Washington (Smith 1939) and *Picea* and *Pinus* in the Canadian Rockies (Schalkwijk 1991). Moser (1960) recognized *C. multiformis* var. *coniferarum* (as *Phlegmacium multiforme* var. *coniferarum*), with *Picea excelsa* and *Pinus*, up to subalpine elevations in Germany, and var. *multiformis* with *Fagus* throughout Europe, with the exception of the Alps. He also refers to material from the Greater Yellowstone Area as *C. multiformis* var. *coniferarum* (Moser *et al.* 1995b). Our material fits the descriptions of Moser (1960) and Smith *et al.* (1979).

Material examined: Mt. Rae: krummholz zone 09 19 97 (ALTA 10189); subalpine forest 08 16 97 (ALTA 10190); 09 19 97 (ALTA 10191). Mt. Tripoli: subalpine forest 08 15 95 (ALTA 10192).

Cortinarius muscigenus Peck, Ann. Rep. N.Y. State Mus. 41: 71. 1888. (subgenus *Myxacium*)

A circumboreal conifer associate. Known from northern Europe in nutrient-poor *Picea* forests (Brandrud *et al.* 1990, Hansen and Knudsen 1992). Also in coniferous forests in New York, New England and New Jersey (Kauffman 1932) and Washington (Smith 1944). Under *Abies*, New York (Smith *et al.* 1979). Synonymous with *C. cylindripes* Kauffman *fide* Bendiksen *et al.* (1992). *C. muscigenus* may also be conspecific with *C. collinitus* Fr. (Bendiksen *et al.* 1992, Brandrud *et al.* 1990), but nomenclatural problems make the interpretation of *C. collinitus* difficult. Not previously reported from Canada.

Material examined: Mt. Rae: subalpine forest 08 16 97 (ALTA 10212); 09 19 97 (ALTA

10213). Smith Dorrian Valley, Kananaskis: 08 17 97 (ALTA 10214).

Cortinarius orichalceus Fr., Epicr. Syst. Mycol., p. 267. 1838. var. *olympianus* A.H. Smith, Lloydia 7: 184. 1944. (subgenus *Phlegmacium*)

A montane conifer associate. *C. orichalceus s.l.* is associated with *Picea excelsa* in Europe (Moser 1960), in mountainous areas of Britain with conifers or *Fagus* (Orton 1955), in western North American mountains with *Picea engelmannii* and *Abies lasiocarpa* (Kauffman 1921, 1932, Smith 1939, 1944), and in Nova Scotia (Gourley 1983). Known from the Greater Yellowstone Area (Moser *et al.* 1995b). Smith (1944) recognized var. *olympianus* A.H. Smith and var. *xanthocephalus* A.H. Smith from Washington.

Our material has a brick red-brown pileus with a greenish margin. Gills at first yellow green, quickly turning blue after collection. Stipe white with green tones and a reddening, marginate base. Context white with blue, then green, suffusing pigments. Cortina white. In one collection (ALTA 10193), green pigment suffused throughout the sporocarps (including gills and cortina) after collection. Sporocarps drying purple. All parts, including pileus, greenish-yellow in 5% KOH. Spores amygdaliform to citriniform, 9.7-11.6 x 5.5-7.0 (-7.4) μm. This seems closest to var. *olympianus* A.H. Smith, which is also the variety collected in Colorado by Kauffman (Smith 1944). *C. orichalceus s.l.* has not been reported previously in western Canada.

Material examined: Mt. Rae: subalpine forest 09 16 95 (ALTA 10193); 09 19 97 (ALTA 10194). North Kananaskis Pass, AB: 09 21 97 (ALTA 10195).

Cortinarius paragaudis Fr., Monographia, p. 79. 1851. subsp. *oenochelis* Lindstr., *Cortin.*, Fl. Photogr. 2: 33. 1992. (subgenus *Telamonia*)

A northern *Picea* and *Pinus* associate. *C. paragaudis s.l.* is known from northern Finland with *Pinus sylvestris* (Väre *et al.* 1996), Sweden, mainly with *Picea* (Soop 1990) but also *Pinus* (Brandrud *et al.* 1990, Soop 1993), Norway in oligotrophic *Picea* forests (Gulden *et al.*1992), alpine Scandinavia with *Picea excelsa* and (or) *Pinus sylvestris* (Moser 1965) and in the Pacific Northwest and Rocky Mountains (J. Ammirati pers. com. 1998). Moser (1965) separated *C. paragaudis* from the closely related *C. haematochalis* (Bull.: Fr.) Fr. in central Europe and Scandinavia mainly on the basis of spore size and shape. Brandrud *et al.* (1992) further separated *C. paragaudis* from Swedish spruce forests into subsp. *paragaudis* from lowland Sweden, and subsp. *oenochelis* Linst. from northern montane Sweden, with larger spores. Our material fits the descriptions of subsp. *oenochelis* of Soop (1990) and Brandrud *et al.* (1992). Pileus dark red brown, convex, then almost plane with a low umbo and involute margin, up to 6.0 cm in diam., slightly hygrophanous. Lamellae pale grey-brown. Stipe clavate, up to 7 cm long, tapering towards a base of up to 1.5 cm. Universal veil forming greyish-pink belts on stipe, which turn purple in 5% KOH (see Soop 1992). Cortina white. Spores (8.2) 8.8-9.6 (10.0) x 5.7-7.0 μm, Q= 1.25-1.76, **Q**=1.44. *C. paragaudis s.l.* has not previously been reported from Canada.

Material examined: Mt. Tripoli: subalpine forest 08 04 95 (ALTA 10164); 08 15 95 (ALTA 10165). Wildhay River AB: 08 06 95 (ALTA 10166).

Cortinarius percomis Fr., Epicr. Syst. Mycol., p. 260. 1838. (subgenus *Phlegmacium*)

A *Picea* associate. In central and northern Europe, in mixed, boreal and montane conifer forests, mainly with *Picea* on calcareous soils (Moser 1960, 1983, Brandrud *et al.* 1994). In Washington and Colorado, in montane *Picea*, *Abies* and *Tsuga* forests (Kauffman 1921, 1932) and Oregon and California under *Picea* (Smith 1939). Associated with *Picea abies* (Trappe 1962). Not previously reported from Canada.

Material examined: Mt. Rae: subalpine forest 09 29 95 (ALTA 10196); 09 19 97 (ALTA 10197). Mt Tripoli: 09 07 97 (ALTA 10198).

Cortinarius scandens Fr., Epicr. Syst. Mycol., p. 312. 1838. *sensu* Moser. (subgenus *Telamonia*)

A widespread generalist. Found in damp coniferous woods and alder bogs in Europe (Moser 1983), with *Picea* or other conifers in California and Washington (Smith 1939, 1944), montane conifers in Colorado (Kauffmann 1921) and in deciduous and coniferous woods from New York (Adirondaks) to Michigan (Kauffman 1932). Associated with *Pinus sylvestris* or *Salix glauca* (Trappe 1962). Arnold (1993) considers *C. obtusus* Fr. a synonym. *C. obtusus* is widely distributed and has a broad host range (Molina *et al.* 1992); found in northern and central Europe under *Picea*, *Pinus*, *Fagus*, *Salix* and *Betula* into the alpine zone (Brandrud *et al.* 1994, Gulden *et al.* 1992, Moser 1982, Scherfose 1990), but especially with *Picea* (Arnold 1993). Also with *Salix* in the U.K. (Watling 1981) and Greenland (Lange 1957, Petersen 1977). In Alaska with *Salix* and *Betula nana* (Ammirati and Laursen 1982)(as *C.* aff. *obtusus*) and in the Greater

Yellowstone Area (Moser *et al.* 1995b). Smith (1939) recognized *C. obtusus* under *Pinus* and *C. scandens* under *Picea* in California. The ectomycorrhizae of *C. obtusus* have been described on *Picea abies* (Agerer 1987a).

C. scandens has been separated from *C. obtusus* by smaller spores and less robust habit (Moser 1983). Arnold (1993) gives a spore size of (6.5) 8-10 x 5-6 μm for *C. obtusus* Fr. (= *C. scandens* Fr.). Spore size in our material is (6.3) 6.8-7.4 (8.4) x (4.4) 5.0-5.6 μm, and is therefore closer to *C. scandens* described by Kauffman (1932), Moser (1983) and Dahnke (1993). Not previously reported from western Canada.

Material examined: Mt. Tripoli: subalpine forest 08 04 95 (ALTA 10167). Mt. Rae: 09 19 97 (ALTA 10168).

Cortinarius traganus (Fr.:Fr) Fr., Epicr. Syst. Mycol., p. 281. 1838. (subgenus *Sereciocybe*)

A common conifer associate. Frequent and widespread in European coniferous forests under both *Picea* and *Pinus* (Brandrud *et al.* 1994), Norway with *Picea abies* (Gulden *et al.* 1992) and northern Finland with *Pinus sylvestris* (Väre *et al.* 1996). Not uncommon in old growth conifer forests in the Pacific Northwest (Smith *et al.* 1979) and frequent under *Abies* and *Picea* in Nova Scotia (Smith and Wehmeyer 1936). Also known from mixed conifer-deciduous forests (Ammirati *et al.* 1995)

Material examined: Mt. Rae: subalpine forest 08 16 97 (ALTA 10224); 09 19 97 (ALTA 10225).

Cortinarius triformis Fr., Hymen. Eur., p. 382. 1874. (subgenus *Telamonia*)

Associated with conifers, especially *Picea*. In central and northern Europe with *Picea* (Hansen and Knudsen 1992, Arnold 1993) and up to tree line in the Austrian Alps with *Picea*, *Pinus*, and *Larix* (Moser 1982). Also in California with *Quercus* (Arora 1986), in the Greater Yellowstone Area (Moser *et al.* 1995b) and in the Rocky Mountain Foothills with *Picea* (Currah *et al.* 1989). Arnold (1993) proposes moving conifer-associated material to *C. fusco-pallens* (Fr.) N. Arnold, and reserving the name *C. triformis* Fr. for deciduous forest material, even though they are morphologically similar. Our material agrees well with Arnold's (1993) description of *C. fusco-pallens*, but the epithet *triformis* has been retained until we have a better understanding of North American *Telamonia*. Pileus 2.5-6.0 cm in diam., dark red brown, strongly hygrophanous, often with a fatty texture. Lamellae pale grey-brown. Stipe whitish with a bulbous base. Universal veil white, forming a thin, peronate sheath on the stipe. Context dingy white to pale brown. Spores ellipsoid, 8.0-9.5 x (5.0) 5.4-6.3 μm. Sporocarps often caespitose. The faint blue tint at stipe apex described by Arnold (1993) was not noted. Not previously reported from Canada.

Material examined: Mt. Rae: krummholz zone 08 16 95 (ALTA 10169); 09 29 95 (ALTA 10170); 08 16 97 (ALTA 10171); subalpine forest 09 14 96 (ALTA 10172). Mt. Tripoli: subalpine forest 08 4 95 (ALTA 10173); 08 15 95 (ALTA 10174); 08 16 97 (ALTA 10175); 09 07 97 (ALTA 10176). Rocky-Clearwater Forest AB: S. Abbott, 08 20 87 (UAMH M0487).

Cortinarius uraceus Fr., Epicr. Syst. Mycol., p. 309. 1838. (Subgenus *Telamonia*)

A northern conifer associate. Widespread in European coniferous and mixed woods, often with *Picea*, also *Abies*, *Quercus*, and *Fagus* (Arnold 1993, Brandrud *et al.* 1994) or *Pinus* (Kallio and Kankainen 1966, Arnolds *et al.* 1995). Found near timberline in the Austrian (Moser 1982) and Swiss Alps with *Picea* (Favre 1960). Also with montane conifers in Colorado and the Adirondacks (Kauffman 1921, 1932), Washington (Smith 1944) and the Greater Yellowstone Area (Moser *et al.* 1995b). Also in Wyoming, with alpine *Salix* and *Picea engelmannii* (Moser and McKnight 1987) and boreal (Hutchison *et al.* 1988) and southern Québec (Pomerleau 1980). Not previously reported from western Canada.

Material examined: Mt. Tripoli: subalpine forest 08 15 95 (ALTA 10177). Mt. Rae: subalpine forest 08 16 95 (ALTA 10178).

Cortinarius venetus (Fr.) Fr., Hymen. Eur., p. 374. 1874. var. *montanus* Mos., Zeitschr. F. Pilzk. 36: 43. 1970. (subgenus *Leprocybe*).

A *Picea* associate, normally in montane to subalpine habitats (Moser 1983). Associated with *Picea* in the Alps (Moser 1970), Fennoscandia (Høiland 1980, Hansen and Knudsen 1992) and the Netherlands (Arnolds *et al.* 1995). Also in the Greater Yellowstone Area (Moser *et al.* 1995b). *C. venetus* var. *montanus* differs from var. *venetus* in its association with *Fagus*, and in having a darker green pileus (Moser 1970). Our material has bright yellow fluorescence under UV (especially the stipe base), small clavate cheilocystidia and subglobose spores [5.5-7.5 x 4.5-6.0 (6.5) μm, **Q** = 1.20].

Ectomycorrhizae are described on *Picea abies* by Agerer (1987b). Not previously reported from Canada.

Material examined: Mt. Tripoli: subalpine forest 07 25 95 (ALTA 10216); 09 07 97 (ALTA 10217).

Cortinarius zinziberatus (Fr.) Fr., Hymen. Eur., p. 392. 1874. (subgenus *Leprocybe*).

A montane conifer associate. Known from central Europe and the Italian Alps in montane and subalpine forests with *Picea* or with *Fagus* (Moser 1969) and Fennoscandia with *Picea*, fruiting early in the season (Høiland 1980, Hansen and Knudsen 1992). Known from the Greater Yellowstone Area (Moser *et al.* 1995b). *Cortinarius ahsii* McKnight, found with conifers in the central Rockies, is likely *C. zinziberatus* (J. Ammirati pers. comm.1997). Our material fits the descriptions of Moser (1969) and McKnight (1975). Pileus yellow-brown, 2-5 cm in diam. Stipe pale brown, up to 6 cm. Lamellae ± concolorous with pileus. Cortina greenish-yellow becoming orange-red in 5% KOH. Spores ellipsoid to amygdaliform, 6.5-9.5 x 4.5-5.5 μm. Tissue of all collections fluorescing bright greenish-yellow in UV (especially velar tissue, stipe base and basal hyphae). Fruiting soon after snow melt. The violet tint to the young gills and stipe base as described in Høiland (1980) was not observed. Not previously reported from Canada.

Material examined: Mt. Tripoli: subalpine forest 07 18 95 (ALTA 10218); 08 04 95 (ALTA 10219); 09 07 97 (ALTA 10220). Mt. Rae: subalpine forest 07 25 95 (ALTA 10221); 08 16 95 (ALTA 10222); 07 27 96 (ALTA 10223).

***Cortinarius* sp.** (subgenus *Sericeocybe*)

Pileus up to 4 cm in diam., pallid cream to off-white, drying to pale tan, convex, glabrous with faint pale tan fibrils toward margin, merely darkening in 5% KOH. Lamellae pallid tan to dingy white. Stipe up to 6 x 2 cm with base enlarged up to 3 cm, concolorous with pileus. Context whitish, no reaction in 5% KOH. Universal veil white, heavy, forming a boot at stipe base. Cortina white. Spores amygdaliform, strongly verrucose, especially at apex, 9.1-10.8 x 5.7-6.8 μm. This *Cortinarius* seems best placed in the subgenus *Sericeocybe* Orton, based on pileus texture, pallid colours and KOH reaction of the pileipellis. It seems close to *C. traganus*, but has larger spores and lacks the violet colours and unpleasant odour.

Moser (in Singer 1986) states that members of the subgenus *Sericeocybe* may be eventually placed into either *Phlegmacium* or *Telamonia*, and Brandrud *et al.* (1990-94) already treat *Sericeocybe* as a section of *Telamonia*. Once the subgeneric classification of *Cortinarius* has stabilized, this species may be best placed in *Telamonia*.

Material examined: Mt. Rae: subalpine forest 08 16 95 (ALTA 10226); 08 15 97 (ALTA 10227); krummholz zone 08 16 95 (ALTA 10228).

Dermocybe crocea (Schaeff.:Fr.) Moser, Schweiz. Z. Pilzk. 52: 98. 1974: *sensu lato.*

Circumpolar, with conifers or arcto-alpine vegetation. Common in Fennoscandia with lowland and boreal *Picea* and *Pinus* and in low and middle arctic regions with *Betula nana* (Høiland 1984, Brandrud *et al.* 1992, Väre *et al.* 1996). Also in Iceland (Lange 1955)(as *Cortinarius cinnamomeus*), the Faeröes (Möller 1945) (as *C. malicorius*

according to Høiland 1984), Scotland with montane vegetation (Watling 1987), the Swiss Alps with dwarf alpine *Salix* (Graf 1994), and the Rocky Mountain Foothills, with *Pinus contorta* and *Picea glauca* (Currah *et al.* 1989). Ectomycorrhizae have been described on *Pinus sylvestris* by Uhl and Agerer (1987).

Our material [spores 6.4-9.1 x 4.2-5.6 (6.4) μm] agrees well with the concept of Høiland (1984), but Moser (1974) describes smaller sporocarps and smaller spores. North American material appears to constitute an unresolved complex which includes *Dermocybe incognitis* Ammirati and Smith and its western variants (J. Ammirati pers. comm. 1997).

Material examined: Mt. Rae: krummholz zone 08 16 95 (ALTA 10229); 07 25 95 (ALTA 10230); subalpine forest 08 16 97 (ALTA 10231). Mt. Tripoli: subalpine forest 08 04 95 (ALTA 10232); 08 15 95 (ALTA 10233). Rocky Clearwater Forest AB: R. Currah 08 6 86 (UAMH M0131); S. Abbott 08 20 87 (UAMH M0493); 09 02 87 (UAMH M0497).

Hebeloma crustuliniforme (Bull. ex St. Am.) Quél., Flor. mycol. Fr., p. 92. 1888. *sensu lato*.

Cosmopolitan and common with a wide range of coniferous and angiosperm hosts. Often associated with *Corylus* and *Fraxinus* in Europe (Hacskaylo and Bruchet 1972, Bruchet 1970). Ectomycorrhizae have been described on *Abies* (Acsai and Largent 1983) and synthesized *in vitro* on *Picea abies* (Brunner *et al.* 1991), *Pinus virginiana* (Hacskaylo and Bruchet 1972) and *Populus tremuloides* (Godbout and Fortin 1985). *H.*

crustuliniforme is a highly variable and poorly understood taxon.

Material examined: Mt. Tripoli: alpine zone 09 07 97 (ALTA 10279); krummholz zone 09 11 94 (ALTA 10280); 09 07 97 (ALTA 10281). Mt. Rae: subalpine forest 08 16 97 (ALTA 10282); 09 19 97 (ALTA 10283).

Hebeloma insigne Smith, Evenson and Mitchel, Veiled species of *Hebeloma* in western U.S., p 132. 1983.

A montane conifer associate. In *Picea, Abies* and *Pinus* forests in Colorado (Smith *et al.* 1983) and under conifers and *Populus* in Colorado and New Mexico (Arora 1986). *H. insigne* is similar in appearance to *H. sinapizans* (Paul.: Fr.) Gillet, but can be distinguished by dextrinoid spores, vinaceous brown pileus and positive $FeSO_4$ reaction on stipe base (Smith *et al.* 1983). Not previously reported from Canada.

Material examined: Mt. Tripoli: subalpine forest 08 15 95 (ALTA 10284).

Hebeloma cf. subfastigiatum Smith, Evenson and Mitchel, Veiled species of *Hebeloma* in western U.S., p 180. 1983.

Pileus up to 3 cm in diam., red-brown, fading to pale tan, convex, glabrous except for faint brown veil remnants on margin, no reaction in 5% KOH. Lamellae ± concolorous with pileus. Stipe up to 4 x 0.5 cm, fibrous, yellow-brown, with brown velar remains toward the apex, base green in $FeSO_4$. Context buff, taste and smell raphinaceous. Cortina brownish. Spores inequilateral, slightly roughened, slowly

dextrinoid, 9.7-12.4 (13) x (5.1) 5.8-7.1 (8.2) μm. Cheilocystidia abundant, fusoid-ventricose to lageniform, some with extended necks (40-75 x 5.5-7.0 μm, up to 12 μm at base), similar to those of *H. fastibile* (Pers.:Fr.) Kumm. Although Smith *et al.* (1983) describe many *Picea*-specific veiled *Hebelomas* from high elevation western forests, our material seems closest to *H. subfastigiatum*, described from a recently burned-over hardwood forest in Michigan.

Material examined: Mt. Rae: subalpine forest 08 16 95 (ALTA 10285); 08 14 95 (ALTA 10286); Mt. Tripoli: alpine zone 09 07 97 (ALTA 10287).

Inocybe dulcamara (Alb.and Schw.) Kumm., Führ. Pilzk., p 79. 1871. *sensu lato.*

Widespread and common in arctic and alpine habitats, generally with dwarf *Salix* and *Dryas*, or with *Betula* or *Populus* at lower elevations or latitudes. Reported from the French, Swiss and Austrian Alps (Favre 1955, Horak 1960, Senn-Irlet 1988, 1993), Fennoscandia (Lange and Skifte 1967, Jacobsson 1984), the Netherlands (Arnolds *et al.* 1995), Greenland (Petersen 1977), the Faeröes (Möller 1945) Iceland (Larsen 1932), Scotland (Watling 1981, 1987), Washington with subalpine *Abies* and *Pinus* (Stuntz 1947), California (Nishida 1989) and in Canada in arcto-alpine or boreal habitats (Malloch 1973, Miller 1987, Hutchison *et al.* 1988). Favre (1955) recognized six forms of *I. dulcamara,* which should be regarded as a species complex. Our material fits the description of Malloch (1973). Pileus up to 4.5 cm in diam., ochraceous, finely fibrillose. Stipe up to 4 cm, concolorous with pileus or paler, pruinose at apex, becoming hollow, without an abrupt bulb but base may be slightly

enlarged. Cortina whitish. Lamellae ochraceous, yellow marginate. Context dingy buff, without distinctive smell. Spores smooth, phaseoliform to broadly ellipsoid, (8.0) 9.0-10.8 x 5.0-6.7 μm. Cheilocystidia numerous, clavate to pyriform, 15-30 x 8-20 μm. Pleurocystidia absent.

Material examined: Mt. Rae: krummholz zone 08 16 95 (ALTA 10288). Mt. Tripoli: alpine zone 08 04 95 (ALTA 10311); krummholz zone 08 04 95 (ALTA 10312); subalpine forest 09 11 94 (ALTA 10313); 08 15 95 (ALTA 10314); 08 30 97 (ALTA 10315); 09 07 97 (ALTA 10316).

Inocybe flocculosa (Berk.) Sacc., Syll. Fung. 5: 768. 1887.

A cosmopolitan generalist. Widespread in Europe and North America, associated with *Betula, Salix, Populus, Alnus, Quercus, Fagus, Picea* and *Pinus* (Kuyper 1986). Known from the Swiss Alps, with dwarf alpine *Salix* (Favre 1955), and from Greenland with *Betula*. Also with conifers in California (Nishida 1989) and in subalpine forests of mixed *Picea, Abies* and *Pinus* in Washington (Stuntz 1947) and Colorado (Kauffman 1921). Also reported from arctic Canada (Dearness 1923) and Québec (Pomerleau 1980). Our material corresponds well to Stuntz's (1947) description of *Inocybe lucifuga* (Fr.) Quél. from a subalpine *Abies-Pinus* forest in Washington, which is now considered a yellow variant of *I. floccosus* (Kuyper 1986). Not previously reported from western Canada.

Material examined: Mt. Tripoli: subalpine forest 08 15 95 (ALTA 10317); 09 07 97 (ALTA 10318); krummholz zone 09 11 94 (ALTA 10319).

Inocybe lacera (Fr.:Fr.) Kumm., Führ. Pilzk. p. 79. 1871.

A cosmopolitan generalist. Widespread in Europe and North America, not uncommon in subalpine and alpine areas, associated with *Betula, Castanea, Salix, Quercus, Alnus, Picea* and *Pinus* (Kuyper 1986, Arnolds *et al.* 1995). With *Salix* in the French and Swiss Alps (Kühner and Lamoure 1986, Favre 1955, Graf 1994), Scotland (Watling 1987), and the Faeröes (Möller 1945). In Greenland and Norway with *Betula* (Petersen 1977, Lange 1957, Gulden and Lange 1971) and with *Pseudotsuga menziesii* (Jansen 1991), *Picea abies* (Bieri 1995), or *Pinus sylvestris* (Scherfose 1990, Termorshuizen 1991, Väre *et al.* 1996) in northern Europe. Found throughout North America in various habitats but especially under *Populus* in northern U.S. and Canada (Kauffman 1924, Smith *et al.* 1979). Ectomycorrhizae of *I. lacera* have been described on *Populus tremuloides* (Cripps and Miller 1995). This taxon is extremely variable, especially arctic and alpine material. Forms such as *I. lacera* var. *heterosperma* Grund and Stuntz fit within the normal range of variation of *I. lacera* (Kuyper 1986). Not previously reported from western Canada.

Material examined: Mt. Tripoli: alpine zone 08 16 95 (ALTA 10320); krummholz zone 08 04 95 (ALTA 10321).

Inocybe lanuginella (Schroet.) J. E. Lange, Dansk Bot. Arkiv 2: 45. 1917.

An alpine *Dryas* associate. Reported from Switzerland with *Dryas* (Favre 1955)(as *Inocybe decipiens* and *I. decipientoides*), Norway at subalpine elevations (Gulden and Lange 1971), and Denmark (Lange 1917), Greenland and Lapland with

Salix (Lange 1957). Horak (1987) maintains that *I. lanuginella* (as *Astrosporina lanuginella*) is specific to *Dryas* on calcareous soil, which brings the material collected with *Salix* into doubt. Synonymous with *Inocybe decipientoides* Peck and *I. globocystis* Vel. *fide* Horak (1987). Our material was collected with *Dryas octopetala*. Not previously reported from Canada.

Material examined: Mt. Rae: alpine zone 08 16 95 (ALTA 10322).

Inocybe rimosa (Bull.:Fr.) Kumm., Führ. Pilzk. p. 79. 1871.

A cosmopolitan generalist. Widespread in Europe and North America, associated with a wide range of coniferous and angiosperm hosts including alpine vegetation (Kuyper 1986). Common throughout the Alps (Favre 1955, Kühner and Lamoure 1986, Graf 1994, Senn-Irlet 1988), Scandinavia (Gulden and Lange 1971, Jacobsson 1984), Scotland (Watling 1987), Greenland (Lange 1957, Petersen 1977) and Svalbard (Ohenoja 1971) with *Salix* and *Dryas*. Also associated with *Pinus sylvestris* in Germany (Scherfose 1990), with conifers or *Quercus* in California (Nishida 1989) and throughout the U.S. and Canada in a wide variety of habitats (Kauffman 1924, Grund and Stuntz 1981). Synonymous with *I. fastigiata* (Schiff.: Fr.) Quél. *fide* Kuyper (1986).

Material examined: Mt. Tripoli: krummholz zone 08 04 95 (ALTA 10323). Mt. Rae: subalpine forest 09 07 97 (ALTA 10324).

Inocybe whitei (B. and Br.) Sacc., Syll. Fung. 5: 790. 1887.

A western conifer associate. Widespread in central Europe, usually with *Picea* or *Pinus* but also rarely with deciduous trees (Kuyper 1986, Arnolds *et al.* 1995). Known from California with conifers or *Quercus* (Nishida 1989) and from mixed forests in Nova Scotia (Gourley 1983) and British Columbia (Bandoni 1977) (both as *I. pudica*). Very common with conifers on the west coast of North America but rare east of the Rockies (Smith *et al.* 1979, Grund and Stuntz 1981). Synonymous with *I. pudica fide* Kuyper (1986). Not previously reported from Alberta.

Material examined: Mt. Tripoli: krummholz zone 09 11 94 (ALTA 10325); 09 07 97 (ALTA 10326).

Thaxterogaster pingue Sing. and Smith, Brittonia 10: 211. 1958.

A montane conifer associate; under *Abies lasiocarpa* as well as *Pinus* and *Pseudotsuga* spp. from the Pacific Northwest to Colorado and California (Singer and Smith 1958). Especially at higher elevations, under *Picea* in the Rockies, *Abies* in the Sierra Nevada and Cascade Mountains (Arora 1986) and under mixed conifers in British Columbia (P. Kroeger pers. comm. 1998). The genus *Thaxterogaster* has not been previously reported from Alberta.

Material examined: Mt. Rae: subalpine forest 09 19 97 (ALTA 10349).

Gomphidiaceae

Gomphidius largus Miller, Mycologia 63:1159. 1971.

A montane conifer associate. Under *Picea engelmannii* in Idaho, and *Populus*

and *Picea* spp. in New Mexico, at high elevations (up to 3,000 m)(Miller 1971). Distinguished from *G. glutinosus* (Fr.) Fr. by the presence of inflated cellular gill trama. Gill trama cells in our material are up to 43 μm wide. Not previously reported from Canada.

Material examined: Mt. Rae: subalpine forest 08 17 97 (ALTA 10378).

Hydnaceae

Hydnum repandum Fr., Syst. Mycol. 1: 400. 1821.

Cosmopolitan, with a broad host range (Molina *et al.* 1992). In Europe with deciduous trees or *Pinus* or *Picea* up to subalpine elevations (Breitenbach and Kränzlin 1986, Scherfose 1990, Gulden *et al.*1992). Associated with *Picea abies, Pseudotsuga menziesii*, *Fagus* and *Corylus* (Trappe 1962). Widely distributed throughout the U.S. and Canada (Smith and Smith 1973); under *Picea engelmannii* and *Abies lasiocarpa* in Colorado (Kauffman 1921), *Quercus* and *Pinus* in southern Oregon (Kauffman 1929) and *Picea glauca* and *Populus* in central Alberta (Schalkwijk 1989, 1991, Currah *et al.* 1989).

Material examined: Mt. Rae: subalpine forest 09 29 95 (ALTA 10278).

Hysterangiaceae

Hysterangium separabile Zeller, Mycologia 33: 201. 1941.

Hypogeous under conifers, deciduous trees and shrubs. Common in the mountains of western North America but widely distributed (Smith and Smith 1973).

Known from Europe, South America, New England, New York, Arizona, California, Oregon and Wyoming (Zeller 1941). With conifers and *Quercus* in California (Arora 1986) and *Tsuga canadensis, T. heterophylla* and *T. mertensiana* in the Pacific Northwest (Kropp and Trappe 1982). Under *Pinus, Populus, Pseudotsuga, Alnus* and (or) *Picea* spp. in the Rocky Mountain foothills (Currah *et al.* 1989).

Material examined: Mt. Rae: subalpine forest 07 21 95 (ALTA 10198); 08 16 95 (ALTA 10379).

Russulaceae

Lactarius alnicola A. H. Smith, Brittonia 12: 319. 1960.

A western conifer associate. Known from Michigan, California, Oregon, Idaho and Wyoming (Hesler and Smith 1960). Common in the northern Rockies (Hesler and Smith 1979) and their foothills with mixed *Picea* and *Pinus contorta* (Currah *et al.* 1989). Originally collected under *Alnus,* but now recognized as a conifer associate (Hesler and Smith 1979). Ectomycorrhizae on *Picea engelmannii* are described by Kernaghan *et al.* (1997).

Material examined: Mt. Tripoli, subalpine forest 09 11 94 (ALTA 9870); 08 30 97 (ALTA 10248); 09 07 97 (ALTA 10249).

Lactarius caespitosus Hesler and Smith, N. Am. sp. *Lactarius*, p. 349. 1979

An *Abies* associate. Common in the spruce-fir zone of the Rocky mountains, rare west of the crest of the Cascade Mountains (Hesler and Smith 1979).

Ectomycorrhizae described on *Abies lasiocarpa* by Kernaghan *et al.* (1997) are probably restricted to that host. Not previously reported from Canada.

Material examined: Mt. Rae: subalpine forest 09 24 94 (ALTA 9871); 08 16 95 (ALTA 10250); 09 29 95 (ALTA 10251); 09 19 97 (ALTA 10252); krummholz zone 08 16 95 (ALTA 10253). Mt. Tripoli: subalpine forest 09 11 94 (ALTA 10254); 08 15 95 (ALTA 10255); 09 07 97 (ALTA 10256); krummholz zone 08 04 95 (ALTA 10257).

Lactarius deliciosus var. *areolatus* A. H. Smith, Brittonia 12: 135. 1960.

A western conifer associate. In Europe, *L. deliciosus s.l.* (an unresolved complex) is normally found with *Pinus* (Hesler and Smith 1979, Arnolds *et al.* 1995). In North America, *L. deliciosus s.l.* has been reported from a wide range of habitats, including Nova Scotia with *Picea* and *Abies* (Smith and Wehmeyer 1936), boreal forest and tundra in northern Québec (Hutchison *et al.* 1988) and northern Alberta with *Pinus banksiana* (Visser 1995). Ectomycorrhizae have been synthesized on *Larix occidentalis, Picea sitchensis, Pseudotsuga menziesii, Tsuga heterophylla* and a number of *Pinus* spp. (Molina and Trappe 1982). *L. deliciosus* var. *areolatus* is the most common variant in the west and is abundant in the Rocky Mountains; reported from Alaska to New Mexico under mixed conifers (Hesler and Smith 1979). Ectomycorrhizae on *Abies lasiocarpa* have been described by Kernaghan *et al.* (1997). This variety has not been previously reported from western Canada.

Material examined: Mt. Rae: krummholz zone 08 16 95 (ALTA 10258); 09 19 97

(ALTA 10259); subalpine forest 08 16 95 (ALTA 10260); 09 19 97 (ALTA 10261). Mt. Tripoli: krummholz zone 08 05 94 (ALTA 9872).

Lactarius luculentus Burl., Mycologia 28: 260. 1936. *sensu lato.*

A western conifer associate. Under *Pseudotsuga menziesii* in Oregon, Colorado and Alaska (Burlingham 1936, Hesler and Smith 1979). Kauffman's (1921) report of *L. subdulcis* under *Pinus* in Colorado may have been *L. luculentus*, because *L. subdulcis* is a similar European species not recognized from North America in the most recent monograph (Hesler and Smith 1979). Hesler and Smith (1979) describe *L. luculentus* var. *luculentus* and var. *latetus* Hesler and Smith from North America. These fungi are part of a complex, which also includes *L. mitissimus* (Fr.) Fr. and *L. aurantiacus* Fr., of small, reddish orange *Lactarii* with white unchanging latex (or latex which becomes yellow on white paper). Our material has glabrous, papillate, dark apricot coloured pilei, slightly paler towards margin, up to 3 cm in diam. Pileus cuticle an ixotrichoderm, lacking incrusting material. Stipe concolorous, very finely fibrillose, stuffed becoming hollow, ± equal, up to 3.5 cm. Lamellae slightly paler, slightly decurrent. Context whitish, taste slightly acrid. Latex scant, white, unchanging. No parts reacting in 5% KOH. Spores (6.5) 7.0-8.5 (9.5) x 5.5- 6.7 (7.3) μm, ornamentation a broken reticulum with isolated warts. Not previously reported from Canada.

Material examined: Mt. Rae: krummholz zone 09 19 97 (ALTA 9875); subalpine forest 09 19 97 (ALTA 10262). Mt. Tripoli: subalpine forest 09 11 94 (ALTA 10263).

Lactarius pubescens Fr., Epicr. Syst. Mycol., p. 335. 1838.

Associated with *Betula* and members of the Salicaceae. Reported from northern Europe with *Betula* (Kallio and Kankainen 1966, Jacobsson 1984, Arnolds *et al.* 1995), Greenland with *Betula* or *Salix* (Lange 1957, Knudsen and Borgen 1982), Alaska with *Betula nana* and *B. papyrifera* (Miller 1982) and *Salix alaxensis* (Laursen and Ammirati 1982) and central Alberta with *Betula papyrifera* (Schalkwijk 1989). Common throughout the boreal forest (Rendall 1980). Mycorrhizae have been synthesized *in vitro* with *Populus tremuloides* (Godbout and Fortin 1985). Hesler and Smith (1979) also recognize *L. pubescens* var. *betulae*, with yellowing latex and smaller spores than var. *pubescens*.

Material examined: Mt. Tripoli: krummholz zone 08 05 94 (ALTA 9874); 09 11 94 (ALTA 10264); 08 15 95 (ALTA 10265); 08 30 97 (ALTA 10266); 09 07 97 (ALTA 10267).

Russula brevipes Peck, 43rd Rep. N.Y. St. Mus. p. 20. 1890.

Common and widely distributed, mainly with conifers but also with angiosperms. Also in arctic and alpine habitats. Known from northeastern North America with *Abies, Picea, Pinus, Populus* and *Betula* (Shaffer 1964, Stanis 1979, Pomerleau 1980), Alaska (Kobayashi *et al.* 1967), the Pacific Northwest with *Pseudotsuga, Abies, Picea, Pinus, Tsuga* (Trappe 1962, Shaffer 1964), and Alberta with *Picea glauca* (Schalkwijk 1989, Currah *et al.* 1989). Ectomycorrhizae described on *Abies lasiocarpa* (Kernaghan *et al.* 1997). Shaffer (1964) describes *R. brevipes* var.

acrior Shaffer, which has a blue-green tint to stipe apex and (or) lamellae and a similar distribution to var. *brevipes*. *Russula delica* Fr. is a similar species commonly reported from Europe in temperate areas with *Pinus, Quercus*, *Fagus* and other deciduous trees, also with montane *Picea* and in alpine regions with *Dryas* (Romagnesi 1967, Einhellinger 1987, Arnolds *et al.* 1995). It is unclear if *R. delica* and *R. brevipes* are conspecific, but Shaffer (1964) suggests rejecting the name *R. delica* and using *R. brevipes* for North American material.

Material examined: Mt. Tripoli: krummholz zone 09 11 94 (ALTA 9878); 08 04 95 (ALTA 10235).

Russula integra (Vitt.) Fr., Epicr. Syst. Mycol., p. 360. 1838.

A montane conifer associate, restricted to Pinaceae (Molina *et al.* 1992). Common in central and northern Europe in montane and subalpine *Picea-Abies* forests, or with *Pinus sylvestris,* also at lower elevations in mixed forests (Romagnesi 1967, Einhellinger 1987, Hansen and Knudsen 1992). Reported with *Pseudotsuga* in California (Arora 1986) and *Abies* in Nova Scotia (Smith and Wehmeyer 1936). Mycorrhizae described on *Abies lasiocarpa* (Chapter Four of this thesis). Not previously reported from western Canada.

Material examined: Mt. Tripoli: krummholz zone 09 11 94 (ALTA 9880); 08 04 95 (ALTA 10236); 09 07 95 (ALTA 10237); subalpine forest 09 11 94 (ALTA 10238); 08 04 95 (ALTA 10239).

Russula silvicola Shaffer, Beih. Nova Hedwigia 51: 229. 1975.

Associated with coniferous and deciduous trees, often with *Abies*. Known from eastern North America in deciduous, coniferous or mixed forests (Shaffer 1975); from Ontario and Québec under *Abies, Picea* and mixed deciduous trees (Nantel and Neumann 1992, Stanis 1979). Ectomycorrhizae described on *Abies lasiocarpa* (Kernaghan *et al.* 1997). *R. emetica* (Schaeff.:Fr.) Pers. ex S.F. Gray var. *silvestris* Sing., which may be conspecific (see Shaffer 1975), is known from Europe with *Pinus sylvestris, Picea abies* and in mixed forests (Einhellinger 1987, Kühner and Romagnesi 1953) and Colorado (Kauffman, 1921). Not previously reported from western Canada.
Material examined: Mt. Rae: krummholz zone 08 16 95 (ALTA 10240); 09 19 97 (ALTA 10241); subalpine forest 09 14 96 (ALTA 10242); 09 19 97 (ALTA 10243). Mt. Tripoli: krummholz zone 08 05 94 (ALTA 9881); subalpine forest 09 11 94 (ALTA 10244); 09 07 97 (ALTA 10245).

Russula torulosa Bres., Iconogr. Mycol. 9: 433. 1929.

A conifer associate, usually with *Pinus*. Known from central and northern Europe with *Pinus*, more rarely under *Picea* (Romagnesi 1967, Hansen and Knudsen 1992, Arnolds *et al.*1995). Also from Washington with *Tsuga mertensiana* and *Pseudotsuga menziesii* (Grund 1965). Moser and McKnight (1987) report the closely related *R. queletii* from tree line in Wyoming with *Picea engelmannii*. Not previously reported from Canada.
Material examined: Mt. Rae: krummholz zone 09 24 94 (ALTA 9877); 09 29 95

(ALTA 10246); 09 07 97 (ALTA 10247).

Scutigeraceae

Albatrellus flettii Morse ex Pouz., Česka Mycol. 26: 198. 1972.

A western conifer associate. Known from Alaska, Alberta, British Columbia, California, Idaho, New Mexico, Oregon, Washington and Wyoming, typically with *Tsuga,* but *Abies, Picea, Pinus, Pseudotsuga, Taxus, Alnus* and *Salix* also reported in the vicinity (Gilbertson and Ryvarden 1986, Ginns 1997). Restricted to the Pinaceae (Molina *et al.* 1992) and associated with *Tsuga heterophylla* (Kropp and Trappe 1982).

Material examined: Mt. Rae: subalpine forest 08 16 95 (ALTA 10380).

Thelephoraceae

Boletopsis subsquamosa (Fr.) Kotlaba and Pouz., Česka Mycol. 11: 164. 1957.

A circumboreal conifer associate. Known from northern U.S. and Canada, uncommon (Gilbertson and Ryvarden 1986). Reported from Nova Scotia with *Picea* and *Pinus* (Gourley 1983). Ectomycorrhizae described on *Picea abies* by Agerer (1992), [as *B. leucomelaena* (Pers.: Fr.) Fayod].

Material examined: Mt. Rae: krummholz zone 08 12 94 (ALTA 10354); subalpine forest 09 24 94 (ALTA 10355); 08 16 95 (ALTA 10356); 09 14 96 (ALTA 10357); 09 19 97 (ALTA 10358).

Hydnellum caeruleum (Hornem.) Karst., Medd. Soc. Fauna Fl. Fenn. 5: 41. 1879.

Associated with conifers in the north and broadleaved trees in the south. Known from Europe with *Pinus sylvestris* (Scherfose 1990, Väre *et al.* 1996, Arnolds *et al.* 1995) or in mixed forests (Breitenbach and Kränzlin 1986). In California with Quercus, *Lithocarpus* and *Arbutus* (Arora 1986) and in Florida (Baird and Kahn 1986). Also common in northern conifer forests (Nova Scotia, Québec), especially with *Pinus* (Harrison 1961, 1968, Pomerleau 1980), and in Alberta with *Picea glauca* and *P. engelmannii* (Currah *et al.* 1989, Schalkwijk 1991). Mycorrhizae described on *Picea engelmannii* (Chapter Four of this thesis).

Material examined: Mt. Rae: subalpine forest 08 12 94 (ALTA 10272); 09 24 94 (ALTA 10273); 08 16 97 (ALTA 10274); 09 19 97 (ALTA 10275).

Hydnellum suaveolens (Scop.:Fr.) Karst., Mcdd. Soc. Fauna Fl. Fenn. 5: 41. 1879.

A northern and montane conifer associate. Known from Europe with montane *Picea* (Breitenbach and Kränzlin 1986) and in North America from New Mexico to British Columbia and from the Appalachian Mountains to Nova Scotia (Harrison 1968, Kauffman 1921). With *Picea abies* in North Carolina (Baird 1986) and with *Picea engelmannii* and *Abies lasiocarpa* in Alberta (Schalkwijk 1991).

Material examined: Mt. Tripoli: krummholz zone 08 05 94 (ALTA 10277).

Pseudotomentella tristris (Karst.) M.J. Larsen, Nova Hedwigia 22: 613. 1971.

Widely distributed throughout the northern Hemisphere (Kõljalg 1996). In North America, from British Columbia to New Mexico and New Jersey (Larsen 1971).

Commonly fruiting on a wide variety of decaying coniferous and deciduous wood (Larsen 1971, Ginns and Lefebvre 1993, Kõljalg 1996).

Material examined: Mt. Tripoli: subalpine forest ALTA 09 06 97 (ALTA 10276).

Sarcodon scabrosus (Fr.) Karst., Rev. Mycol. 3: 20. 1881.

Widespread, associated with conifers in the north and broadleaved trees in the south. In Switzerland and the Netherlands with *Fagus, Quercus* and *Castanea* and in Scandinavia with *Pinus* (Breitenbach and Kränzlin 1986, Arnolds *et al.* 1995). With *Liriodendron* and *Quercus* in southeastern North America (Baird 1986, Baird and Khan 1986), conifers or hardwoods in California (Arora 1986), conifers in Nova Scotia (Harrison 1961), *Tsuga heterophylla* in the Pacific Northwest (Hall and Stuntz 1972) and *Picea glauca* in the Rocky Mountain foothills (Currah *et al.* 1989).

Material examined: Mt. Rae: subalpine forest 09 19 97 (ALTA 10268).

***Sarcodon* sp.**

Sporocarps gregarious, solitary or fused, pileus 2-15 cm in diam., pale argillaceous when young, then pale tan to greenish yellow, older parts olivaceous, bruising purple, concave, undulating to nearly plane, with persistently incurved margin, matted fibrillose at first, soon forming coarse scales with dark vinaceous brown edges, slightly olivaceous in 5% KOH. Teeth 3-17 mm in length, crowded, gray with paler tips, bruising lavender from the tip, decurrent to halfway down the stipe, strongly olivaceous in 5% KOH. Stipe 5-9 x 1.5-4.5 cm, central or eccentric, often

fused, ± equal with pointed base, pale orange, lower portion olivaceous, bruising purple, olivaceous in KOH. Context, white, fibrous, no reaction in KOH. Spores small, tuberculate, 3.7-5.0 x 2.8-3.6 μm. These collections appear similar to *S. versipellis* (Fr.) Quél. but differ in colour, length of teeth and bruising reaction.

Material examined: Mt. Rae: subalpine forest 09 29 95 (ALTA 10269); 08 18 95 (ALTA 10270). Mt. Tripoli: krummholz zone 09 06 97 (ALTA 10395); subalpine forest 09 11 94 (ALTA 10271).

Thelephora caryophyllea Fr., Syst. Mycol. 1: 430. 1821.

A north temperate species, on sandy soil under conifers (Corner 1968). Known from Finnish Lapland with *Pinus* and *Betula* (Kallio and Kankainen 1964), the Netherlands with *Pinus* or *Picea* (Arnolds *et al.* 1995), Germany with *Pinus sylvestris* (Scherfose 1990) and the Swiss Alps with dwarf alpine *Salix* spp. (Senn-Irlet 1988). In Colorado with montane conifers (Kauffman 1921), central Alberta under *Populus tremuloides* (Schalkwijk 1989) and northern Alberta with *Pinus banksiana* (Visser 1995).

Material examined: Mt. Tripoli: subalpine forest 08 15 95 (ALTA 10392).

Tomentella ellisii (Sacc.) Jülich and Stalpers, Verh. K. Ned. Akad. Wet. 74: 236. 1980.

Distributed throughout the temperate northern hemisphere (Jülich and Stalpers 1980). Commonly fruiting on a wide variety of decaying coniferous and deciduous

wood (Kõljalg 1996, Ginns and Lefebvre 1993). Not previously reported from Alberta. Some of our collections were from alpine soil supporting *Dryas octopetala*.

Material examined: Mt. Rae: alpine zone 08 16 97 (ALTA 10393); 09 19 97 (ALTA 10396). Mt. Tripoli: subalpine forest 09 07 97 (ALTA 10395).

Tomentella sublilacina (Ellis and Holw.) Wakef., Mycologia 52: 931. 1960.

Distributed throughout the temperate northern hemisphere (Larsen 1974, Jülich and Stalpers 1980). Commonly fruiting on a wide variety of decaying coniferous and deciduous woods (Larsen 1974, Ginns and Lefebvre 1993, Kõljalg 1996). Mycorrhizal with *Pinus muricata* in California (Gardes and Bruns 1996). Reported from Kootenay National Park, British Columbia, on deciduous wood and from Banff National Park, Alberta, on coniferous wood (Larsen 1975, as *T. kootenaiensis* Larsen). Our collections were from alpine soil supporting *Dryas octopetala*.

Material examined: Mt. Rae: alpine zone 08 16 97 (ALTA 10394); 09 19 97 (ALTA 10397).

Tricholomataceae

Camarophyllus pratensis (Fr.) Kumm., Führ. Pilzk., p. 117. 1871.

Cosmopolitan, often in open grassy areas or forest edges (Breitenbach and Kränzlin 1991) but also in thickets or dense forests (Hesler and Smith 1963, Smith *et al.* 1979, Bird and Grund 1979). Reported with subalpine *Betula* in Sweden (Jacobsson 1984).

Material examined: Mt. Tripoli: subalpine forest 08 15 95 (ALTA 10342); 08 30 97 (ALTA 10381); 09 07 97 (ALTA 10382). Mt. Rae: subalpine forest 08 16 95 (ALTA 10344); 09 19 97 (ALTA 10383).

Catathelasma imperiale (Fr. in Lund) Sing., Rev. Mycol. 5: 9. 1940.

A circumboreal conifer associate. Known from Europe, Asia and the west and east coasts of North America, mainly with *Picea,* but also *Pseudotsuga, Tsuga, Abies* and rarely *Pinus* (Hutchison 1992). Restricted to Pinaceae (Molina *et al.* 1992). **Material examined**: Mt. Rae: subalpine forest 09 24 94 (ALTA 10367); 09 29 95 (ALTA 10368); 09 14 96 (ALTA 10369); 09 19 97 (ALTA 10370). Mt. Tripoli: krummholz zone 09 08 96 (ALTA 10371); subalpine forest 09 07 97 (ALTA 10372). Rocky-Clearwater Forest AB: R. Currah, 08 06 86 (UAMH 5451).

Hygrophorus chrysodon (Fr.) Fr. Epicr. Syst. Mycol., p. 320. 1838.

Widely distributed in North America, associated with conifers and angiosperms. Common in western North America but apparently rare in the east (Hesler and Smith 1963); with *Lithocarpus* and *Arbutus* in coastal California (Arora 1986), *Pseudotsuga menziesii* in the Pacific Northwest (Trappe 1962), montane conifers in Colorado (Kauffman 1921), *Betula nana* and *Dryas octopetala* in Alaska (Miller 1982) and *Picea* or *Pinus* spp. in Alberta (Currah *et al.* 1989, Danielson 1984, Visser 1995). **Material examined**: Mt. Rae: krummholz zone 09 24 94 (ALTA 10327); 09 29 95 (ALTA 10328); 09 19 97 (ALTA 10329); subalpine forest 09 19 97 (ALTA 10329).

Mt. Tripoli: subalpine forest 09 11 94 (ALTA 10330); 08 15 95 (ALTA 10331); 09 07 97 (ALTA 10332).

Hygrophorus erubescens (Fr.) Fr. Epicr. Syst. Mycol., p. 322. 1838.

Circumpolar, usually with *Picea*. Restricted to Pinaceae (Molina *et al.* 1992). In Fennoscandia and Switzerland with *Picea*, especially at montane to subalpine elevations (Breitenbach and Kränzlin 1991, Gulden *et al.*1992, Hansen and Knudsen 1992, Bieri 1995). In northern U.S. and Canada with *Pinus*, *Picea* and *Tsuga* (Hesler and Smith 1963, Smith *et al.* 1979, Bird and Grund 1979); in Alberta under *Picea glauca*, *Pinus contorta* (Currah *et al.* 1989, Schalkwijk 1991) and *Pinus banksiana* (Visser 1995).

Material examined: Mt. Tripoli: subalpine forest 09 11 94 (ALTA 10333); 08 15 95 (ALTA 10334); 09 07 97 (ALTA 10335). Mt. Rae: subalpine forest 09 19 97 (ALTA 10336).

Hygrophorus korhonenii Harmaja, Karstenia 25: 42. 1985.

A circumboreal *Picea* associate. Known from Fennoscandia and Québec, always with *Picea* (Harmaja 1985, Hansen and Knudsen 1992). Harmaja (1985) states that *H. olivaceoalbus* (Fr.) Fr. *sensu* Hesler and Smith does not fit the European concept of *H. olivaceoalbus*, but does fit the description of *H. korhonenii*. *H. olivaceoalbus sensu* Hesler and Smith is widely distributed in northern and western North America as well as France and the former U.S.S.R. with *Picea* (Hesler and Smith 1963). Distinctive

features of *H. korhonenii* include a gelatinous outer veil covering a dark, fibrillose, inner veil, dark fibrillose veil remnants on the sitpe, spores measuring 10-14 x 5.2-7.5 μ m and a northern distribution. Not previously reported from western Canada, but has probably been collected and referred to as *H. olivaceoalbus sensu* Hesler and Smith, e.g. Schalkwijk (1991).

Material examined: Mt. Rae: subalpine forest 09 29 95 (ALTA 10337); 09 19 97 (ALTA 10338); krummholz zone 09 29 95 (ALTA 10339); 09 19 97 (ALTA 10340). Mt. Tripoli: subalpine forest 08 30 97 (ALTA 10341).

Hygrophorus pudorinus (Fr.) Fr., Epicr. Syst. Mycol., p. 322. 1838.

A circumboreal conifer associate. Often with *Abies* in Europe (Kühner and Romagnesi 1953, Breitenbach and Kränzlin 1991, Arnolds *et al.* 1995), but with *Picea* in Sweden (Hansen and Knudsen 1992) and the former U.S.S.R. (Hesler and Smith 1963). Under *Picea*, *Abies* or *Tsuga* in northern and western U.S. and Canada (Smith *et al.* 1979, Bird and Grund 1979, Arora 1986). With *Picea engelmannii, Abies lasiocarpa* and *Pinus contorta* in Colorado (Kauffman, 1921), in boreal forest in northern Québec (Hutchison *et al.* 1988) and *Picea* and *Pinus* in the Canadian Rockies (Schalkwijk 1989).

Material examined: Mt Tripoli: subalpine forest 09 11 94 (ALTA 10343). Mt. Rae: subalpine forest 09 19 97 (ALTA 10345).

Hygrophorus pustulatus (Fr.) Fr., Hymen. Eur., p. 411. 1874.

A widespread conifer associate. Throughout European *Picea* forests, up to subalpine elevations (Breitenbach and Kränzlin 1991, Arnolds *et al.* 1995). Under *Abies* and *Sequoia* in Michigan, Colorado, Wyoming, Idaho, California and Washington (Hesler and Smith 1963) and with *Picea* in Nova Scotia (Bird and Grund 1979). Not previously reported from western Canada.

Material examined: Mt Rae: subalpine forest 09 19 97 (ALTA 10346)

Hygrophorus speciosus Peck, N.Y. State Mus. Ann. Rept. 29: 43. 1878.

A circumboreal *Larix* associate (Hesler and Smith 1963). Known from montane to subalpine Switzerland (Breitenbach and Kränzlin 1991), up to tree line the Austrian Alps with *Larix decidua* (Moser 1982) and with *Larix laricina* in Nova Scotia (Bird and Grund 1979) and Québec (Hutchison *et al.* 1988, Pomerleau 1980). Reported from the southwest U.S. with *Pinus ponderosa* (Arora 1986). Not previously reported from western Canada or in association with *Larix lyallii*.

Material examined: Mt. Rae: krummholz zone 07 25 95 (ALTA 10347); subalpine forest 09 19 97 (ALTA 10348).

Laccaria montana Sing., Sydowia 7: 89. 1973.

Circumpolar and alpine, with conifers and angiosperms. Restricted to arctic, boreal or montane habitats; Switzerland, the Faeröes, Colorado, Idaho, Montana, Washington, Wyoming, Alaska, and British Columbia with members of the Pinaceae, *Betula* and *Salix* (Mueller 1992). Also reported from Iceland and Norway (including Svalbard) (Hansen

and Knudsen 1992) with *Salix* (Gulden 1988). Not previously reported from Alberta. **Material examined**: Mt. Tripoli: krummholz zone 08 04 95 (ALTA 10384); 08 15 95 (ALTA 10385); 09 07 97 (ALTA 10386); subalpine forest 07 18 94 (ALTA 10387). Mt. Rae: alpine zone 08 16 95 (ALTA 10388); subalpine forest 08 16 95 (ALTA 10389).

Tricholoma myomyces (Fr.) Lange, Dansk. Bot. Ark. 8: 21. 1933.

A widespread conifer associate. In northern Europe, often with *Pinus* (Hansen and Knudsen 1992, Arnolds *et al.* 1995) or on sand dunes with *Pinus* and *Salix* (Moser 1983). Under planted *Picea* and *Pinus* in the Great Lakes region with variations found throughout northern U.S. and Canada (Smith *et al.* 1979); in Sweden, Michigan, Minnesota, Pennsylvania, Wisconsin, and Ontario under most species of *Pinus* and *Picea* as well as *Tsuga canadensis* (Ovrebo 1980, 1989). In Alberta with *Picea* and *Pinus* (Currah *et al.* 1989).

Material examined: Mt. Tripoli: subalpine forest 08 15 95 (ALTA 10288); 08 04 95 (ALTA 10289); 09 07 97 (ALTA 10290). Mt. Rae: subalpine forest 07 25 95 (ALTA 10291); 09 19 97 (ALTA 10292).

Tricholoma saponaceum (Fr.) Staude, Schwämme Mitteldtsch., p. 127. 1858.

Widespread, associated with conifers and angiosperms. Known from northern Europe with *Picea* (Gulden *et al.* 1992), *Pinus sylvestris* (Väre *et al.* 1996), *Fagus* or *Quercus* (Arnolds *et al.* 1995). In northeastern North America with *Abies, Picea, Tsuga* or *Pinus* spp. (Ovrebo 1980), northern Alberta with *Pinus banksiana* (Visser 1995), Nova

Scotia with *Picea* (Smith and Wehmeyer 1936), Colorado with *Picea engelmannii* and *Pinus contorta* (Kauffman 1921), central Alberta with *Populus tremuloides* (Schalkwijk 1989) or *Pinus contorta* (Currah *et al.* 1989), and California with *Lithocarpus* and *Arbutus* (Arora 1986).

Material examined: Mt.Tripoli: subalpine forest 09 11 94 (ALTA 10293); 09 07 97 (ALTA 10294). Mt. Rae: krummholz zone 09 24 94 (ALTA 10295); subalpine forest 07 21 94 (ALTA 10296); 07 25 95 (ALTA 10297); 08 16 95 (ALTA 10298); 08 16 97 (ALTA 10299).

Tricholoma vaccinum (Pers.: Fr.) Staude, Schwämme Mitteldtsch., p. 128. 1858.

A circumboreal *Picea* associate, also reported with *Pinus*. In central and northern Europe with *Picea* up to subalpine elevations (Breitenbach and Kränzlin 1991, Hansen and Knudsen 1992, Bieri 1995). Widely distributed in northern U.S. and Canada with *Picea* and (or) *Pinus* (Smith *et al.* 1979, Ovrebo 1980); with *Pinus* in northern California (Arora 1986), *Picea glauca* in N.W.T (Bigelow 1959) and *Picea glauca, Picea engelmannii* and *Pinus contorta* in the Canadian Rockies (Currah *et al.* 1989, Schalkwijk 1991). Ectomycorrhizae are described on *Picea abies* by Agerer (1987b).

Material examined: Mt. Rae: subalpine forest 09 29 95 (ALTA 10300); 09 19 97 (ALTA 10301); 09 19 97 (ALTA 10302). Mt. Tripoli: subalpine forest 09 11 94 (ALTA 10303); 09 07 97 (ALTA 10304).

Tricholoma virgatum (Fr.) Kumm., Führ. Pilzk., p. 134. 1871. var. *vinaceum* Ovrebo and

Tylutki, Mycologia 67: 79. 1975.

Probably a montane conifer associate. *T. virgatum* var. *virgatum* is common in Europe with conifers and *Fagus* (Bon 1984) and throughout the northern forests of North America with *Pinus* and *Picea* (Ovrebo 1989). *T. virgatum* var. *vinaceum*, also a conifer associate, has a vinaceous flush to the stipe with age or upon bruising, and is known from Idaho, Oregon and Washington (Ovrebo 1975). Bon (1984) describes *T. virgatum fo. rosipes* Bon, also with a reddening stipe, growing in Europe with montane conifers. Given this, and the locations of the North American collections, it is likely that *T. virgatum* var. *vinaceum* is a montane variety. In our collections all but lamella tissue became vinaceous upon handling. *T. virgatum* var. *vinaceum* has not been previously reported from Canada. **Material examined**: var. *vinaceum* - Mt. Rae: subalpine forest 09 24 94 (ALTA 10305); 09 29 95 (ALTA 10306); 09 19 97 (ALTA 10307); krummholz zone 09 24 94 (ALTA 10308). Mt. Tripoli: subalpine forest 09 11 94 (ALTA 10309); 09 07 97 (ALTA 10310). Priest Lake Idaho: C. Ovrebo, 11 05 72 (CO 90). Var. *virgatum* - Idaho: E. Tylutki (EET 6600). Muskoka Dist., Ontario: L. Hutchison 19 09 86 (LH-96-86); 09 17 94 (LH-93-94). Devon, Alberta: S. Abbott (M0272, M05576, M0366).

Discussion

Vegetation at the alpine-subalpine ecotone in the Canadian Rockies is a mosaic of gymnosperms and low growing angiosperms and combines species from the subalpine forest with those from the alpine zone. Obligately ectomycorrhizal host genera include *Abies, Larix, Picea, Salix, Dryas* and *Betula*, as well as *Kobresia* and *Polygonum* to a

lesser extent. This is twice the number of common ectotrophic host genera of either of the adjacent habitats and is comparable to the number found at lower elevations in Alberta (see Moss 1983). Only at this ecotone, however, do they occur together in close proximity.

As the specificity of ectomycorrhizal fungi often operates at the level of host genus (Molina and Trappe 1982), we might expect the community of ectomycorrhizal fungi at tree line to reflect vegetational patterns and exhibit greater richness than in the adjacent habitats. Although ectomycorrhizal fungi at the ecotone included both associates of subalpine gymnosperms and alpine angiosperms (Table 2.1), richness was not positively correlated with that of the host plants, but instead decreased steadily with elevation. This pattern is likely due to the rich subalpine community combining with the more depauperate alpine community to give an intermediate richness at the ecotone. This is in contrast to the subalpine and alpine plant communities, which are more similar to each other in species number, and combine to give the relatively high plant richness seen at the ecotone.

The overall decrease in ectomycorrhizal sporocarps across tree line fits the general trends of decreasing species diversity (Chapin and Körner 1995) and decreasing fructification (Gardes and Dahlberg 1996) with elevation. Comparisons of ectomycorrhizae across tree line reveal a similar pattern with increasing elevation (Kernaghan and Currah, unpublished data) Lower richness in the alpine zone may be due to less amenable climatic and edaphic factors, particularly soil temperature and moisture (Peredo *et al.* 1983, Vogt *et al.* 1992), less carbon production from dwarf vegetation (Newman 1988) and (or) fewer ectomycorrhizal symbioses with angiosperms than with

gymnosperms (Richardson 1970, Bieri *et al.* 1992).

Of the species collected, approximately 86% are also known from Europe, while only 14% appear to be endemic to North America (i.e. not found in the European literature). If we delineate North American varieties, the percentages are 84 and 16, respectively. The amount of overlap varies considerably among genera, however, with *Lactarii* representing mainly North American taxa, and *Russula* representing mainly species with circumboreal distributions. Among the *Cortinarii* collected, only *C. clandestinus* (and possibly the unidentified *Cortinarius*) is endemic to North America. This is in contrast to the 50% similarity between European and North American *Cortinarius* species estimated by M. Moser (pers. comm. 1994).

It is possible that the lack of endemic species in genera such as *Russula* and *Cortinarius* is a reflection of the lack of North American monographic treatments of these genera, and that in time, more endemics will be delineated. It is also possible that with more years of collecting, more endemic species may have been encountered. Arnolds (1992) estimates that with monthly visits, 80% of macrofungi would be seen in four years and Richardson (1970) continued to see new species in the fifth year of intensive monitoring of small plots. Our results appear similar, with 13 taxa (16%) being collected for the first time during the fourth year. Four of these, however, were either hypogeous, secotioid or resupinate, and may have been simply overlooked in previous years.

It seems more likely however, that the low level of endemism found during this study is due to the dominance of *Picea* and *Salix* as ectomycorrhizal hosts at tree line. The

occurrence of these genera throughout the taiga and arcto-alpine zones allows for the circumboreal or circumpolar distribution of many of the fungi collected. At lower elevations or latitudes, e.g. coastal British Columbia, higher levels of vascular plant endemism are encountered (Pojar 1993). With less floristic similarity to European forests, we would expect these regions to exhibit less overlap in species of ectomycorrhizal fungi.

Species richness of ectomycorrhizal fungi was not higher at the alpine/subalpine ecotone as might have been expected on the basis of ectotrophic host genera richness. The documentation of ectomycorrhizal fungi from this zone has however, extended many distributional ranges and revealed many new associations.

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Table 2.1. Habitats and probable hosts of taxa collected. Hosts are determined from literature review and location of sporocarps. + = literature report of symbiosis, ? = possible association.

	Probable host(s)					
Fungal species	*Betula*	*Dryas*	*Salix*	*Abies*	*Larix*	*Picea*
Alpine only						
Inocybe lanuginella		+				
Tomentella sublilacina		+	?			
Alpine and krummholz						
Cortinarius albonigrellus			+			
C. favrei			+			
C. galerinoides			+			
C. inops		+	+			
Inocybe lacera	+		+	?		+
Krummholz only						
Amanita vaginata	+		+			
Cortinarius clandestinus				?		?
C. evernius	+		+	?		+
C. hinnuleus		+	+			
Hydnellum suaveolens				?		+
Inocybe whitei				?		+
Lactarius pubescens	+					
Russula brevipes	+	+		+		+
R. torulosa						+
Krummholz and forest						
Boletopsis subsquamosa						+
Catathelasma imperiale				+		+
Cortinarius delibutus	+		+	?		+
C. fulminoides						+
C. multiformis				+		+
C. triformis				?		+
Cortinarius sp.				?		?
Dermocybe crocea	+		+			+
Hygrophorus chrysodon						+
H. korhonenii						+
H. speciosus					+	
Inocybe flocculosa	+		+	?		+
I. rimosa		+	+	+	+	+
Lactarius caespitosus				+		
L. deliciosus				+	+	+

Fungal species	Probable host(s)					
	Betula	*Dryas*	*Salix*	*Abies*	*Larix*	*Picea*
L. luculentus				?		?
Russula integra				+		?
R. silvicola				+		?
Sarcodon sp.				?		?
Suillus aeruginascens					+	
Tricholoma saponaceum				+		+
T. virgatum				?		+
Forest only						
Albatrellus flettii				?		?
Camarophyllus pratensis				?		?
Cortinarius brunneus						+
C. calochrous				?		+
C. chrysomallus						+
C. colus				?		+
C. crassus				?		+
C. dilutus						+
C. glaucopus				?		+
C. muscigenus				+		+
C. orichalceus				+		+
C. paragaudis						+
C. percomis						+
C. scandens				+		+
C. traganus				?		+
C. uraceus				+		+
C. venetus						+
C. zinziberatus				?		+
Gomphidius largus						+
Hebeloma insigne				?		?
Hydnellum caeruleum						+
Hydnotrya cubispora				?		+
Hydnum repandum				?		+
Hygrophorus erubescens						+
H. pudorinus				+		+
H. pustulatus				+		+
Hysterangium separabile				?		?
Lactarius alnicola				?		+
Pseudotomentella tristis				?		?
Rhizopogon rubescens				+	+	+
Sarcodon scabrosus				?		+
Suillus cavipes					+	
Thaxterogaster pingue				+		+

Fungal species	Probable host(s)					
	Betula	*Dryas*	*Salix*	*Abies*	*Larix*	*Picea*
Thelephora caryophyllea				?		+
Tricholoma myomyces				?		+
T. vaccinum						+
Alpine and forest						
Amphinema byssoides	?	+	+	+	?	+
Cenococcum geophilum	+	+	+	+	+	+
Laccaria montana	+		+	+		+
Hebeloma crustuliniforme	+	+	+	+		+
H. cf. subfastigiatum		?	?	?		?
Inocybe dulcamara	+	+	+	?		?
Tomentella ellisii	?	+	?	?	?	?

Figure 2.1. Map of southern Alberta showing locations of collecting sites. Darkened areas represent montane habitats.

Chapter Three

Russulaceous ectomycorrhizae of *Abies lasiocarpa* and *Picea engelmannii*[1]

Introduction

Abies lasiocarpa (Hook.) Nutt. and *Picea engelmannii* Parry form an extensive band of near climax forest in the subalpine zone of the central Rocky Mountains. In the Front Range of the Canadian Rockies, these species reach their upper elevational limit between 1,900 and 2,450 m, where they become stunted and multi-stemmed, taking on a krummholz growth form (Baig 1972). In these high elevation environments, plants are especially dependant on mutualistic symbioses with mycorrhizal fungi to aid in water and nutrient acquisition (Moser 1967, Haselwandter 1987, Väre et al. 1997). In spite of this, subalpine ectomycorrhizae have received little attention in Europe (Moser 1982, Treu 1990) and even less in North America (Cázares 1992).

During a study of the ectomycorrhizal communities in the subalpine forests of the Front Range of the Canadian Rockies we have found the Russulaceae to be common and conspicuous conifer associates. Ectomycorrhizae formed by species of *Lactarius* and *Russula* are relatively distinctive, and several species of Russulaceous fungi have been shown to form mycorrhizae with members of the genus *Picea* (Alexander 1981, Molina and Trappe 1982, Agerer 1986, Treu 1990, Amiet and Egli 1991, Weiss 1991, Pillukat and Agerer 1992, Kraigher et al. 1995), while only two Russulaceous species, *Lactarius*

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deliciosus Fr. (Acsai and Largent 1983) and *Russula ochroleuca* (Pers.) Fr. (Pillukat and Agerer 1992), have been described from *Abies*. In general, *Abies* has received little attention as a mycorrhizal host, with relatively few mycorrhizae described on the genus (Masui 1926, Acsai and Largent 1983, Kottke and Oberwinkler 1990, Peña-Cabriales and Valdés-Hgo 1974, Pillukat and Agerer 1992). With respect to western North American subalpine conifers, there have been no published descriptions of ectomycorrhizae from either *Picea engelmannii* or *Abies lasiocarpa*.

Until recently, the identification of the fungal taxa in ectomycorrhizal symbioses has been determined by *in vitro* re-synthesis or by demonstrating that hyphae are continuous between mantle tissue and sporocarps. The polymerase chain reaction, in combination with fungus specific primers, now makes identification of ectomycorrhizal fungi from root tips faster and more reliable (Gardes and Bruns 1993, 1996, Erland et al. 1994, Egger 1995). RFLP analysis of PCR amplified ribosomal DNA allows for the comparison of ectomycorrhizae with sporocarps collected at different sites and times, including herbarium specimens (Bruns et al. 1990).

In this study then, we use PCR amplified fungal DNA in conjunction with mycorrhizal anatomy to identify and describe associations between the Russulaceae (*Russula* and *Lactarius*) and the erect and krummholz forms of *Abies lasiocarpa* and *Picea engelmannii*.

Materials and methods

Collection

Ectomycorrhizae were collected from the organic soil horizon in 3.5 cm diam. soil cores taken down to mineral soil. Samples were collected each month during the snow-free season of 1994 along transects through the subalpine forest and the krummholz zone at Mt. Tripoli and Mt. Rae. For detailed site descriptions, see Kernaghan and Currah in press, or Chapter Two of this thesis.

Soil cores were placed in sections of plastic pipe to maintain their integrity during transport. Russulaceous sporocarps were collected monthly from both sites during 1994, 1995 and 1996. Soil cores and sporocarps were stored on ice until examined.

Morphological characterization and photography

Sporocarps were identified, air-dried and deposited in the University of Alberta Cryptogamic Herbarium (ALTA). Ectomycorrhizae were separated from soil by wet sieving samples through a 850 μm soil sieve placed over a 600 μm sieve. The morphology of ectomycorrhizae deemed to be Russulaceous (based on the presence of smooth mantles, laticifers, cystidia or other distinctive cells and the absence of clamp connections) was described using a dissecting photomicroscope with fibre optic lighting. Host identity was determined either by tracing the root system from the mycorrhizae to the tree or by analysis of cross-field pitting in attached secondary root tissue (Core et al. 1979).

Anatomical descriptions were made using the oil immersion objective of an Olympus photomicroscope with bright field and Nomarsky interference. For light

microscopy, portions of fungal mantle were peeled from root tissue and mounted in 5% KOH, and in Ponceau-S (Daughtridge et al. 1986). Fresh mantle tissue was also mounted in sulphovanillin (Singer 1986) which gives a dark blue colour reaction in laticifers and other specialized cells of Russulaceous mycorrhizae (Miller et al. 1991) (designated here as S.V. +). This reaction occurred in fresh material but not in mycorrhizae that had been frozen.

Scanning electron microscopy (SEM) used a Jeol JSM6301FXV equipped with a Emitek K1200 cryo system. Samples were quick-frozen in liquid nitrogen slush, placed on cryo-sublimation stage for 10 min at -40^0 C and then placed on the SEM cold stage at -40^0 C and examined at low voltage until surface ice had been removed. Samples were then cooled to -155^0 C and transferred to a cryo-chamber for gold sputtering and then returned to the SEM cold stage for examination at 2.5 kV. Images were digitally edited in Adobe Photoshop 3.0.

DNA extraction and amplification

DNA was extracted from one or two sporocarps of each of the nine Russulaceous species collected on the subalpine sites, as well as from five herbarium specimens characteristic of spruce-fir forests. DNA was also extracted from fresh, frozen or lyophilized subsamples of Russulaceous ectomycorrhizae. Total genomic DNA was extracted from 2-5 mg lyophilized ectomycorrhizae (10-25 mg if fresh or frozen), and 5 mg dried lamellae by the method outlined by Gardes and Bruns (1993). When amplification (see below) was unsuccessful, DNA was extracted following Jobes et al.

(1995) modified by using 5% of the recommended amounts of tissue and reagents.

Amplification protocols were modified from Gardes and Bruns (1993). DNA extracts were diluted 1:25, 1:125, 1:625 and 1:3125 with water and a 25 μL aliquot of each dilution was added to an equal volume of PCR cocktail containing 400 μM of each dNTP (Canadian Life Technologies Inc., Burlington, ON), 100 mM KCl, 5 mM MgCl, 20 mM Tris-HCl (pH 9), 2.5% Triton X-100 (Sigma-Aldrich Canada, Mississauga, Ont.), 1 unit *Taq* DNA polymerase (Promega Corp., Madison, WS.) and 2 μM of each oligonucleotide primer; ITS1-F (Gardes and Bruns 1993) and ITS4 (White et al. 1990). Temperature cycling used a Techne Gene-E programable thermocycler as follows: 70^0 C while DNA was added to cocktail, 94^0 C for 2 min, then 30 cycles of 94^0 C for 1 min 55^0 C 1 min and 72^0 C for 2 min and finally 72^0 C for 10 min.

RFLP analysis

10 μL aliquots of PCR amplified product were combined with 7μ L water, 1 μL restriction endonuclease; either *Alu*I, *Hha*I, *Hinf*I or *Rsa*I (Pharmacea Biotech inc., Baie D'Urfe, PQ) and 2 μL buffer (supplied by manufacturer). These restriction enzymes recognize 4 bp sequences common in fungal ITS (Egger 1995). After 3-5 hours at 37^0 C, the digested DNA was loaded onto 2% agarose gels (ICN Biomedicals, Aurora, OH) and separated by electrophoresis for 4 hours at 75 V in a 1% TBE buffer (90 mM Tris-borate, 1 mM EDTA, pH 8.3).

Mycorrhizal and sporocarp DNA, digested by the same enzyme, were loaded side by side for comparison and a 123 bp DNA ladder (Sigma, St. Louis, MO) used to

determine fragment size. Gels were stained with ethidium bromide and viewed under UV light. Images were captured with a Mitsubishi gel documentation system and analysed with Gel Pro analyzer software (Media Cybernetics, Silver Springs, MD).

Results

All Russulaceous taxa from the subalpine sites and other locations (Table 3.1) could be differentiated by RFLP analysis of the amplified ITS region (Table 3.2). Russulaceous ectomycorrhizae from the subalpine sites were matched (Fig. 3.1, Table 3.2) with *Lactarius alnicola* Smith, *L. caespitosus* Smith & Hesler, *L. deliciosus* Fr. var. *areolatus* Smith, *Russula brevipes* Peck and *R. silvicola* Shaffer and are described in detail below. Fragment sizes given (Table 3.2) are accurate to ± 3%. Fragments of less than 75 bp could not be resolved.

The size of the ITS regions amplified varied among the genera analyzed (Table 3.2); from 720 bp in *Russula* (slightly larger in *R. nigricans*) to 738 in *Macowanites* and between 746 and 775 bp in *Lactarius*.

The restriction enzymes also varied in their ability to cleave the ITS region. On average *Rsa*I cleaved less frequently than the other enzymes (average number of resulting fragments: *Rsa*I =1.36, *Alu*I = 2.01, *Hha*I = 2.21, *Hinf* I = 2.21). Although *Rsa*I recognized no restriction sites in some species, e.g. *L. caespitosus, L. pubescens* and *R. xerampelina* (Table 3.2, *L. caespitosus* in Fig. 3.1, lanes 8 & 9), the data was still informative and differentiated *L. caespitosus* from *L. deliciosus* var *areolatus*.

In some cases (*L. caespitosus* and *L. deliciosus*) *Hinf*I cleaved the ITS region into

two equal fragments (Table 3.2, *L. caespitosus* in Fig. 3.1, lanes 6 & 7). The two fragments then overlapped on the gel resulting in a band with twice the normal fluorescence.

Descriptions of ectomycorrhizae

Lactarius alnicola (on *Picea engelmannii*) (Figs. 3.2, 3.3)

Monopodial-pinnate to pyramidal. Mantle smooth, yellow-ochre (concolorous with sporocarp). Hyphal strands (50-100 µm wide) differentiated into inner, ± parallel, hyaline hyphae (2.5 - 3.5 µm wide) and an outer prosenchymous layer of cells similar in size and shape to those of outer mantle. Emanating hyphae uncommon, hyaline (3.0 - 3.5 µm wide). Outer mantle prosenchymous, cells short, blocky (sometimes rounded), (4.5 - 10 µm wide x 17 - 35 µm long) often with short branches. Cells relatively thick-walled and finely encrusted, containing yellow pigment. Central mantle synnenchymous, cells becoming shorter and thinner walled (6.0 - 9.0 µm wide x 10 - 19 µm long), some forming rosettes, isolated clusters of cells strongly S.V.+. Laticifers abundant, obtusely branched, (4.5 - 10 µm wide) strongly S.V. +. Inner mantle a reticulum formed by obtusely branched, ± cylindrical hyphae (2.0 - 9.0 µm wide).

L. caespitosus (on *Abies lasiocarpa*) (Fig. 3.4)

Monopodial-pinnate. Mantle smooth, covered with a thin layer of mucilaginous material (<1µ), hyaline to olivaceous (similar to pigment suffused throughout the sporocarp). Hyphal strands (30-200 µm wide) olivaceous, differentiated into outer pigmented hyphae

(2.0 - 3.5 µm wide) and inner hyaline hyphae (3.5-6.0 µm wide). Emanating hyphae uncommon (2.5 - 3.5 µm wide). Outer mantle a prosenchyma of elongated, non-interlocking epidermoidal cells (1.5 - 5.0 µm wide x 17- 45 µm long). Laticifers abundant in inner mantle (4.0 - 11 µm wide), obtusely branched, S.V. weakly positive, contents appearing granular in 5% KOH. Inner mantle a tightly woven synnenchyma of narrow, obtusely branched, ± cylindrical hyphae (2.5 - 4.5 µm wide).

L. deliciosus* var. *areolatus (on *Abies lasiocarpa*)

Monopodial-pinnate. Mantle smooth, pale orange, becoming green where bruised or with age (*i.e.*, concolorous with sporocarps). Hyphal strands ± cylindrical (150 - 200 µm wide), pale yellow but soon becoming green, hyphae parallel (2.0 - 3.0 µm wide) but often contorted and wider at surface of larger strands. Emanating hyphae uncommon, similar to inner hyphae of strands. Outer mantle prosenchymous, formed from cylindrical, obtusely branched hyphae (3.0 - 4.5 µm wide), often running parallel and forming narrow sheets. Laticifers common (3.5 - 7.0 µm wide), greenish in sulphovanillin, contents separated by wall material every 4.0 - 10 µm. Inner mantle hyphae becoming shorter, wider and more branched (4.5 - 5.5 µm wide).

R. silvicola (on *Abies lasiocarpa*) (Fig.3.5).

Monopodial-pinnate. Mantle smooth, hyaline (assuming colour of underlying root tissue). Emanating hyphae not seen. Outer mantle composed of subangular/polygonal cells, 3 - 4 sided (some sides rounded) (4.5 - 7.5 µm x 7.0 - 11 µm wide), often forming rosettes of 4

- 5 cells, isolated cells strongly S.V. +, grading through a layer of short, non-interlocking, epidermoidal cells (5.5 - 11 µm wide x 9.0 - 22 µm long) to an inner synnenchymous mantle, composed of narrow, obtusely branched, cylindrical hyphae (1.5 - 4.0 µm wide).

R. brevipes (Figs. 6-8)

Monopodial-pinnate. Mantle fuzzy (due to many erect cystidia), hyaline to fuscous/copper. Emanating hyphae not seen. Outer mantle a loose reticulum of cylindrical hyphae (2.5 - 5.0 µm wide) with 2, 3 and 4 way intersections, giving rise to a dense layer of ampule-shaped (rarely cylindrical) cystidia (3.5 - 6.5 µm wide x 13 - 30 µm long) with 2-3 small, rounded apical projections. Inner mantle synnenchymous, a mixture of cylindrical hyphae and partially interlocking epidermoidal cells (3.0 - 8.0 µm wide x 16 - 38 µm long).

Although no secondary root tissue was collected with the *R. brevipes* mycorrhizae, the morphology of the fine roots, as well as the proximity of host plants, strongly indicates that *A. lasiocarpa* is the host.

Discussion

The symbioses identified in this study are morphologically distinct and common at the subalpine sites studied. We describe them on either *Picea engelmannii* or *Abies lasiocarpa*, but some may be capable of concurrent symbioses with other woody plants (e.g., species of *Larix*, *Betula*, *Salix* or *Dryas*). We expect that these fungi may be recognizable on other hosts as anatomical characters of the mantle and emanating

elements are often determined by the mycobiont (Brand 1991, Pillukat and Agerer 1992).

Lactarius alnicola, now considered a conifer associate (Hesler and Smith 1979), was originally collected under *Alnus,* resulting in its misleading epithet. It has been frequently collected in the Rocky Mountains (Hesler and Smith 1979) where it is associated with *Picea engelmannii* (a subalpine species), and at lower elevations under *Picea glauca* (Moench.) Voss. (Currah et al. 1989). It is then expected that *L. alnicola* forms ectomycorrhizae with *Picea engelmannii* at higher elevations, with *P. glauca* at lower elevations, and with the hybrid of the two (*P. glauca* var. *albertiana* (S. Brown) Sarg.) at intermediate elevations.

The ochraceous-yellow mycorrhizae of *L. alnicola* exemplify the similarity in colouration between the sporocarp and mycorrhizal mantles of all three *Lactarius* species identified. Microscopically, the tissue of the central mantle (Figs. 3.2-3.3) is similar to that of the outer mantle of *Russula silvicola* (Fig. 3.5) in that the cells are highly differentiated and may or may not be sulphovanillin reactive. They differ in shape and in distribution of reactive cells, which are found in isolated clusters in *L. alnicola* and scattered individually throughout the mantle in *R. silvicola.* It is possible that the sulphovanillin reaction in the central mantle cells of *L. alnicola* may be due to latex from damaged laticifers.

L. caespitosus may be endemic to the subalpine zone of the Central Rockies (Hesler and Smith 1979). Because it is not reported from south west British Columbia (Kernaghan personal observation) and is rare west of the Cascade Crest in the United States (Hesler and Smith 1979), its range appears to follow that of *A. lasiocarpa.* This distribution suggests that *L. caespitosus* and *A. lasiocarpa* have a high degree of

host/symbiont specificity.

The narrow, elongate epidermoidal cells of the outer mantle (Fig. 3.4) are characteristic of the genus *Lactarius*, and have also been described from the mycorrhizae of *L. glyciosmus* (Fr.) Fr. (Ingleby et al. 1990), *L. pallidus* (Pers.:Fr.)Fr. (Brand 1991) and *L. vellereus* (Fr.) Fr. (Brand and Agerer 1986).

Lactarius deliciosus s.l. is a widespread and variable conifer associate with five varieties recognized in North America (Hesler and Smith 1979), including var. *areolatus* Smith, which is abundant throughout the Rockies. In North America, mycorrhizae formed by *L. deliciosus s.l.* have been described from nature on *Abies concolor* (Gord. *et* Glend.) Lindl. (Acsai and Largent 1983) and from pure culture synthesis with species of *Larix*, *Picea, Pinus, Pseudotsuga*, and *Tsuga* (Molina and Trappe 1982). Agerer (1986) gives a thorough description of the naturally occurring mycorrhizae of *L. deterrimus* Gröger (= *L. deliciosus* var. *deterrimus* Hesler & Smith) on *Picea abies* (L.) Karst. Although differences between *L. deliciosus* var. *deterrimus* and *L. deliciosus* var. *areolatus* include spore size, degree of zonation of the pileus and intensity of green staining at maturity, the mycorrhizae of the two are morphologically similar.

Russula silvicola is widely distributed throughout North American coniferous forests where it generally fruits in rotten wood or deep humus (Shaffer 1975). The distinctive subangular, sulphovanillin reactive mantle cells have also been described from the mycorrhizae of a similar taxon, *R. emetica* (Schaeff.: Fr) Pers.: Fr. var. *silvestris* Singer on *Fagus* as well as from *R. mairei* Singer on *Fagus, R. nana* Killerm. on *Polygonum* (Brand 1991) and *R. fragilis* (Pers.: Fr.) Fr. on *Picea glauca* (Kernaghan

unpublished), all of which are included in subsection *Emeticinae*, a group delimited in part by the presence of sulphovanillin reactive pileocystidia (Shaffer 1975). Other, more epidermoidal, sulphovanillin reactive mantle cells are described from *R. firmula* Schäffer (subsection *Urentinae*) on *Pinus* and *R. laricina* Velen. (subsection *Laricinae*) on *Larix* (Treu 1990). The sulphovanillin reactive mantle cells of *Russula* mycorrhizae may be analogous to reactive pileocystidia in that they may both act as storage sites of stearyl-velutinal (responsible for the sulphovanillin reaction), a precursor of the toxic antifeedants velleral and isovelleral, also found in the laticifers of *Lactarius* spp. and the cystidia of *Lentinellus* spp. (Camazine and Lupo 1984).

Russula brevipes is widely distributed throughout North America and mainly associated with species of *Abies*, *Picea*, *Tsuga* and *Pseudotsuga* (Stanis 1979). Yamada and Katsuya (1995) describe a symbiosis involving *Russula nigricans* Fr. (also in section *Compactae*) formed *in vitro* with *Pinus densiflora* Sieb.et Zucc. which also has ampule-shaped cystidia with apical projections. Similar cystidia have also been found on the mycorrhizae of *R. illota* Romagn. (section *Ingrate*)(Brand 1991), on the mycorrhizae of *Monotropa* associated with *Russula* species (Martin 1986) and on unidentified mycorrhizae of *Abies firma* Sieb.et Zucc. (Masui 1926).

Comparable cystidia are absent from the sporocarps of *Russula brevipes, R. nigricans* or *R. illota*, and seem more common on *Russula* mycorrhizae than on fruiting bodies. Similar apical projections are described on the more fusiform pileocystidia of *Russula grisea* Gill. var. *iodes* Romagn. (section *Heterophyllae*) (Romagnesi 1967). Although it seems that distinctive structures such as the cystidia of *R. brevipes*, the

sulphovanillin reactive cells of *R.. silvicola* or the elongated epidermoidal cells of *Lactarius caespitosus* should in time prove to be diagnostic features for certain stirps within the Russulaceae, more matches between sporocarps and mycorrhizae are needed.

DNA is especially well suited to the identification of mycorrhizae formed by genera, such as *Russula*, that are difficult to grow in culture (Taylor and Alexander 1989) and possess scanty basal hyphae making *in vitro* resynthesis and hyphal tracing difficult. More specifically, the ITS region has provided the appropriate level of resolution for the taxa studied, as all species tested had distinctive RFLP profiles. This may not have been the case given different taxa however, because RFLP analysis of the ITS region may not discriminate between sibling biological species (Egger 1995).

Intraspecific variation may also be detected, but differs between groups of ectomycorrhizal fungi. No variation in ITS was observed among isolates of *Tylospora fibrillosa* Donk (Erland et al. 1994) and 1-2% variation was seen between isolates of *Laccaria bicolor* (Maire) Ort. (Gardes et al. 1991), while collections of *Cantharellus cibarius* Fr. (and closely related species) showed relatively large length variations (Feibelman et al.1994, 1996). Very few studies involving Russulaceae have utilized DNA analysis and the level of variation in the ITS region is not yet known. Using RFLP analysis of amplified ITS, Kraigher et al. (1995) distinguished between mycorrhizae of *Lactarius lignyotus* Fr. and *L. picinus* Fr. on *Picea abies* and Gardes and Bruns (1996) identified the mycorrhizae of *Russula amoenolens* Romagn., *R. brevipes* and *R.. xerampelina s.l.* on *Pinus muricata* D. Don. Unfortunately, RFLP data from these studies are not directly comparable with ours because of differences in PCR primers.

Given the level of anatomical and biochemical similarity between mycorrhizal mantles and their associated sporocarps, initial identifications, based on anatomical characters with subsequent confirmation using DNA, will expedite ectomycorrhizal identification and allow for the processing of the large sample sizes necessary for ecological investigations.

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Table 3.1. Collecting locations and accession numbers of Russulaceous sporocarps used for RFLP analysis.

Fungus	Location	Accession #
Lactarius alnicola Smith	Mt. Trippoli, AB	ALTA 9870
L. caespitosus Smith and Hesler	Mt. Rae, AB	ALTA 9871
L. deliciosus var. *areolatus* Smith	Mt. Trippoli, AB	ALTA 9872
L. pubescens Fr.	Mt. Trippoli, AB	ALTA 9874
L. affin. *luculentus* Burl.	Mt. Trippoli, AB	ALTA 9875
L. uvidus (Fr.) Fr.	Kananaskis valley, AB	ALTA 9873
Macowanites americana Singer & Smith	Cypress Bowl, BC	ALTA 9876
Russula brevipes Pk.	Mt. Trippoli, AB	ALTA 9878
R. fragilis (Pers.: Fr.) Fr.	Devon, AB	ALTA 9879
R. integra Fr.	Mt. Trippoli, AB	ALTA 9880
R. torulosa Bres.	Mt. Rae, AB	ALTA 9877
R. silvicola Shaffer	Mt. Trippoli, AB	ALTA 9881
R. nigricans Fr.	Prince Albert, SK	ALTA 9882
R. xerampelina Fr.	Devon, AB	ALTA 9883

Table 3.2. Restriction fragment sizes and non-digested ITS region sizes of Russulaceous sporocarps and mycorrhizae. Those not in bold are from sporocarps and those in bold are common between sporocarps and mycorrhizae.

	Enzyme				
Fungus	*Alu*I	*Hha*I	*Hinf*I	*Rsa*I	ITS Region
Lactarius alnicola	**550**	**398**	**368**	**700**	**775**
	219	**361**	**245**	**75**	
L. caespitosus	**536**	**406**	**382**	**764**	**764**
	234	**350**	**382**		
L. deliciosus var. *areolatus*	**529**	**392**	**378**	**670**	**758**
	222	**348**	**378**	**90**	
L. affin. *luculentus*	486	320	364	669	746
	133	222	258		
	81	110	110		
L. pubescens	520	389	382	746	746
	176	316	364		
L. uvidus	503	329	382	694	746
	188	198	364		
		165			
Macowanites americana	460	738	342	661	738
	201		233		
			166		
Russula brevipes	**497**	**364**	**369**	**655**	**720**
	227	**262**	**282**		

R. fragilis	491	363	373	570	720
	227	209	339	149	
		149			
R. integra	441	355	350	680	720
	218	360	340		
R. nigricans	510	412	383	632	727
	214	322	344		
R. silvicola	**481**	**380**	**351**	**514**	**720**
	209	**339**	**271**	**144**	
			96		
Russula torulosa	441	326	347	368	720
	218	272	320	340	
		90			
R. xerampelina	466	354	375	720	720
	203	218	326		

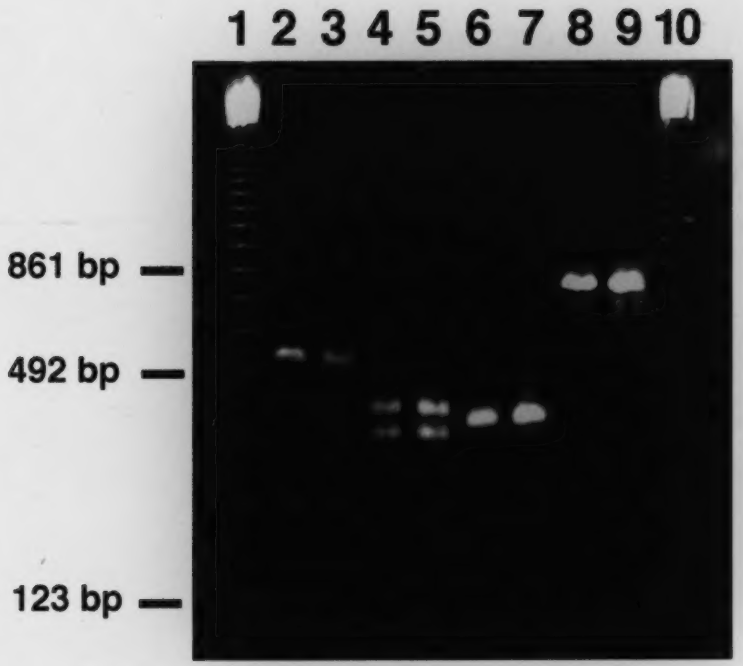
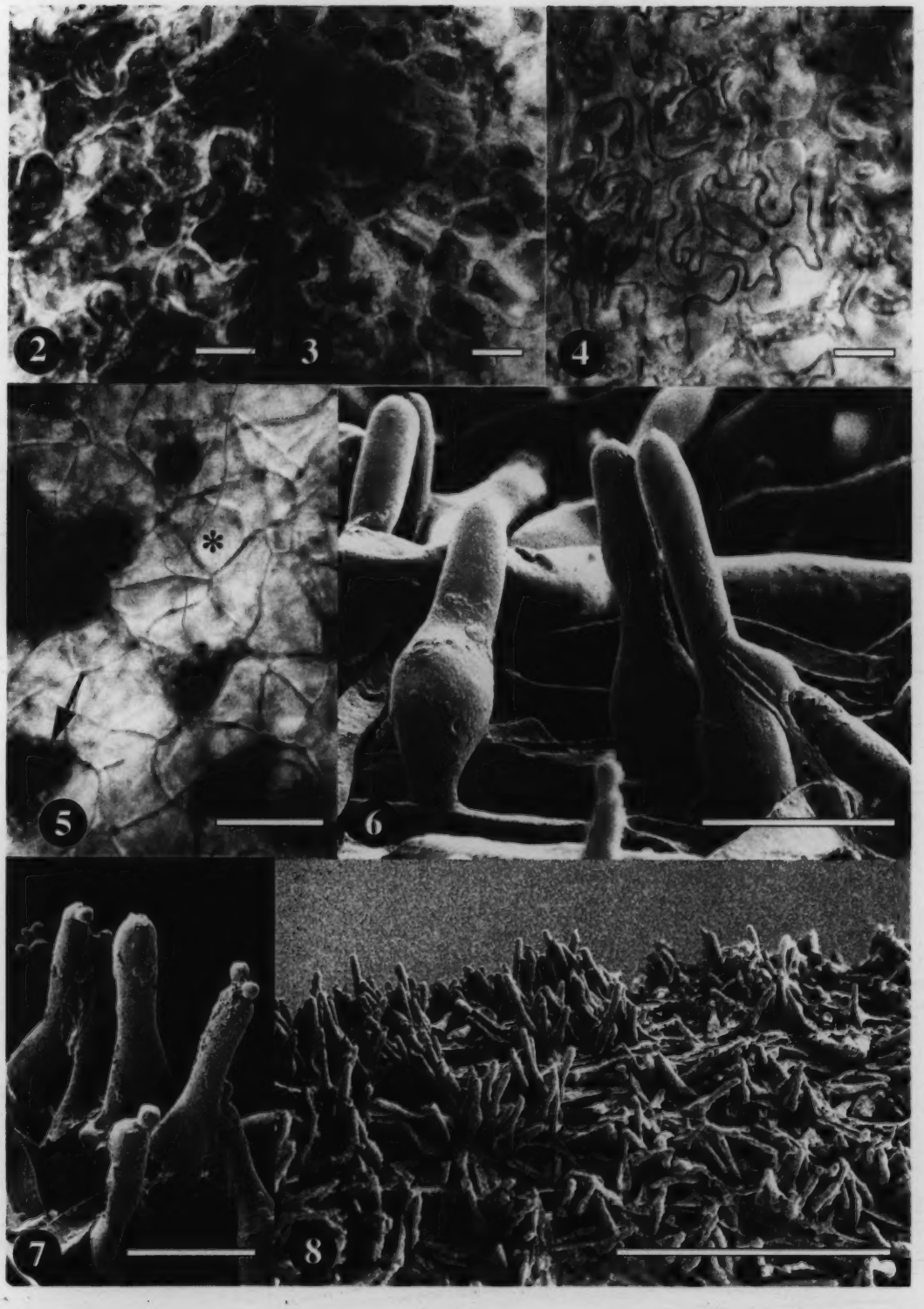


Figure 3.1. RFLP patterns of the ITS region amplified from *Lactarius caespitosus* sporocarp tissue compared to *L. caespitosus* mycorrhizae on *Abies*. Lanes 1 and 10, 123 bp size markers; lanes 2 and 3, treated with *Alu*I; lanes 4 and 5, with *Hha*I; lanes 6 and 7, with *Hinf*I; lanes 8 and 9, with *Rsa*I.

Figs. 3.2-8: Mantle cells of Russulaceous ectomycorrhizae. Figs. 2-3. Subangular cells in central mantle of *L. alnicola*. Scale Bar = 10 µm. Fig. 4. Elongated epidermoidal cells in outer mantle of *L. caespitosus*. Scale Bar = 10µm. Fig. 5. Outer mantle cells of *Russula silvicola* in sulphovanillin, note reactive polygonal cells (arrow) and rosette (asterisk on uppermost cell). Scale Bar = 10µ. Figs. 6-8. Ampule-shaped cystidia on *R. brevipes* mantle. Scale Bar = 10µm in Figs. 6 & 7 and 100 µm in Fig. 8.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Chapter Four

Identification of ectomycorrhizae from montane Alberta[1]

Introduction

Montane habitats are often rich in ectomycorrhizal fungi which are essential for the establishment and support of plants under the stressful conditions found at higher elevations. Sporocarp surveys have been carried out (see Chapter Two of this thesis, Kernaghan and Currah in press), but species composition and relative abundance of fungi forming ectomycorrhizae on montane plants are still poorly understood (Gardes and Dahlberg 1996). Only recently have efforts been made to identify and describe ectomycorrhizae from subalpine forests and adjacent alpine zones e.g., Debaud *et al* 1981, Debaud 1987, Treu 1990, Graf and Brunner 1996, Kernaghan *et al.* 1997.

Identification of the ectomycorrhizae in the above studies has been based on either physical connection between sporocarps and mycorrhizae (see Agerer 1991a), comparison with mycorrhizae synthesized *in-vitro,* (see Fortin *et al.* 1980, Molina and Palmer 1982, Brunner *et al.* 1991), or the comparison of PCR amplified DNA with that of sporocarps (see Gardes and Bruns 1993, 1996a). Other methods for identification include the comparison of cultures obtained from sporocarps and mycorrhizae (Chu-Chou 1979, Chu-Chou and Grace 1983, Danielson 1982, Hutchison 1991) and comparison with published anatomical descriptions (Agerer 1987-1995, Groenbach 1988, Brand 1991, Goodman *et*

[1]Being prepared for submission to Mycorrhiza.

al. 1995-1996, Ingelby *et al.* 1990). PCR based identifications are becoming the methods of choice, however, (Erland *et al.* 1994, Gardes and Bruns 1996b, Kårén and Nylund 1997, Dahlberg *et. al* 1997) because they are fast, reproducible and allow for the comparison of mycorrhizae with sporocarps collected at different times and locations.

In this study, mycorrhizal identification was based on both anatomical and molecular characters. Mycorrhizal anatomy was compared to literature descriptions and to the anatomy of sporocarps collected on the site. Putative identifications were then confirmed by comparing RFLP patterns of mycorrhizal rDNA with those from sporocarps, either directly or by phenetic clustering. This technique allowed for the identification and description of mycorrhizal symbioses from the subalpine and alpine zones of the Front Range of The Canadian Rockies.

Materials and methods

Collection

Ectomycorrhizae and associated sporocarps and were collected in subalpine *Picea engelmannii-Abies lasiocarpa* forests and adjacent alpine zones, between 2,000 and 2,200 m asl on the southeast slope of Mt. Tripoli, in the Nikanassin Range, Alberta (117^0 17'W, 52^0 52'N) and between 2,300 and 2,500 m asl on the southwest slope of Mt. Rae, Peter Lougheed Provincial Park (114^0 59'W, 50^0 36'N). See Chapter Two of this thesis or Kernaghan and Currah in Press for detailed site descriptions. Ectomycorrhizae were collected each month during the snow-free season of 1994 by removing 3.5 cm diam soil cores from the organic soil horizon (down to mineral soil) at 1 m intervals along 16 m

transects. 6 transects were constructed on each site; 2 in the subalpine forest, 2 in the alpine zone and 2 in the transition zone. Soil cores were placed in sections of plastic pipe to maintain their integrity during transport. Selected locations along the transects were re-sampled between 1995-1997.

Ectomycorrhizae were separated from soil by wet sieving samples through a 850 μm soil sieve placed over a 600 μm sieve. The overall morphology was described using a dissecting photomicroscope with fibre optic lighting. Ectomycorrhizae from within each core were then separated into morphological groups which were further divided into 2 parts; with one part frozen in water for subsequent anatomical description and the second part lyophilized for RFLP characterization. Methods used for light and scanning electron microscopy are described in Kernaghan *et al.* (1997). Descriptive terminology is based on Agerer (1987-1995), Ingelby *et al.* (1990), Goodman *et al.* (1995-1996) and Kernaghan *et al.* (1997).

Sporocarps of all putatively ectomycorrhizal fungi, including hypogeous and resupinate taxa, were collected monthly from both sites during 1994-1997 (Chapter Two of this thesis, Kernaghan and Currah in press). Sporocarps were identified, air-dried and deposited in the University of Alberta Cryptogamic Herbarium (ALTA). A representative selection of taxa, including field collected and herbarium material, was used for RFLP analysis. Descriptions of ectomycorrhizae formed by species of *Lactarius* and *Russula* may be found in Kernaghan *et al.* (1997).

DNA amplification and characterization

Total genomic DNA was extracted by grinding up to 5 mg dried lamellae or lyophilized ectomycorrhizae in liquid nitrogen in a ceramic mortar. For mycorrhizae, DNA was extracted from ground tissue using DNeasy Plant Mini Kit (Quiagen, Hilden Ger.) For sporocarps, ground tissue was placed in 500μL CTAB extraction buffer and incubated for 1 hour at 65^0 C. 500 μL chloroform was then added and the mixture centrifuged at 16, 000 g for 15 min. The supernatant was then mixed with isopropanol and centrifuged at 16, 000 g for 15 min. The resulting pellet was then washed with 80% ethanol, dried, resuspended in 60 μL water and cleaned with Glass Max DNA isolation spin cartridge system (Life Technologies, Gaithersburg, MD) prior to amplification. Detailed protocols for the PCR amplification of the ITS region of the nuclear rDNA and for RFLP analysis using the restriction enzymes *Alu*I, *Hha*I, *Hinf*I or *Rsa*I are described in Kernaghan *et al.* (1997).

For the identification of mycorrhizae to species, RFLP fragments from digested mycorrhizal and sporocarp rDNA were run side by side on 2% agarose gels.

Phenetic clustering

In cases where no conspecific RFLP pattern could be found among the sporocarps analyzed, generic identification was made by phenetic clustering, in which taxa are grouped together on the basis of presence or absence of restriction fragments. Restriction fragments produced by each enzyme and non-digested ITS sizes were binned into categories in which fragment sizes varied by no more than 2%. The resulting size

categories were used to construct two species-character matrices; one for members of the Russulaceae, using data from Kernaghan *et al.* (1997), to which was added data for *Hygrophorus pudorinus* and *Tuber rufum,* which may form anatomically similar mycorrhizae, and one for members of the Cortinariaceae, Tricholomataceae and unidentified mycorrhizae. Phenograms were then generated by the neighbor-joining method of Saitou and Nei (1987) using Potemkin software (Brzustowski 1998). Non-digested ITS sizes were given twice the weight of restriction fragment sizes because it was assumed that insertion/deletion events are of greater evolutionary significance than point mutations.

Results

Six ectomycorrhizae were identified to species, 11 to genus or subfamily and 4 remained unidentified. Of these, five are formed by fungi for which mycorrhizae have not previously been described and 2 are new plant-fungus combinations. All 21 are described below.

Phenetic clustering analysis

Phenetic analysis of the RFLP data from the Russulaceae (Kernaghan *et al.* 1997) (Fig. 4.11) resulted in 3 distinct clades; (I) *Russula sensu lato* (including *Macowanites*), (II) *Lactarius* and (III) *Hygrophorus pudorinus* and *Tuber rufum*, included for comparison. The second phenetic analysis, (Fig. 4.12) including members of the Cortinariaceae, Tricholomataceae and unidentified mycorrhizae, resulted in 6 distinct clades; (I)

Cortinarius, subgenera *Myxacium* and *Phlegmacium*, *Dermocybe* and "Forest Cortinarius 1", (II) *Inocybe*, mainly subgenus *Inocybe*, and "Alpine Inocybe" (III) Tricholomataceae including *Tricholoma*, *Laccaria*, *Laccaria* mycorrhizae and "Forest Basidiomycete 2", (IV) other members of the Tricholomataceae, including *Catathelasma*, *Hygrophorus* and "Forest Basidiomycete 1", (V) *Cortinarius*, mainly subgenus *Telamonia* and 3 *Cortinarius* mycorrhizae; "Alpine Cortinarius", "Forest Cortinarius 1" and "Krummholz Cortinarius", and finally (VI) *Inocybe lacera* (subgenus *Inocybe*) and *Inocybe dulcamara* (subgenus *Mallocybe*).

Descriptions of mycorrhizae identified by comparison to published descriptions of mycorrhizae and/or sporocarps

Amphinema byssoides (Pers.:Fr.) Eriksson, on *Abies lasiocarpa, Picea engelmannii, Dryas octopetala* and *Salix barrattiana.* Mantle white to yellow or orange, often very thin, becoming lemon yellow in 5% KOH. Emanating hyphae very abundant, clamped, hyaline to yellow, often papillate. Hyphoid cystidia of sporocarp are absent on mycorrhizae. Very common in both subalpine *Picea-Abies* forest and alpine zone. For more detailed descriptions of ectomycorrhizae formed by *A. byssoides,* see Weiss (1991), Ingelby (1990) and Goodman *et al.* (1996-1997).

Cenococcum geophilum Fr. on *Abies lasiocarpa, Picea engelmannii, Dryas octopetala* and *Salix barrattiana*. Mantle jet black. Emanating hyphae abundant, simple septate, stiff,

often branching at nearly right angles. Hyphae of outer mantle forming a stellate pattern. Associated with smooth black sclerotia up to 3 mm in diam. Very common in both subalpine *Picea-Abies* forest and alpine zone. For more detailed descriptions of ectomycorrhizae formed by *C. geophilum,* see Gronbach (1988), Goodman *et al.* (1996-1997) and Agerer (1987-1995).

Sarcodon sp. (Fig. 4.2) on *Picea engelmannii* and (or) *Abies lasiocarpa*

Monopodial pinnate. Cottony, pale ochre. Emanating hyphae abundant, hyaline to pale yellow, cylindrical, simple septate, often papillate, thin to thick walled, 4.0-11.5 μm wide, often constricted at septa. Outer mantle a disorganized prosenchyma of cylindrical, obtusely branched hyphae, slightly papillate, with strong yellow intracellular pigments 3.6-9.6 μm. wide. Chlamydospores common in outer mantle and on emanating hyphae: globose to subglobose, 5.0-12 μm in diam., roughened to coarsely nodulose, hyaline to yellow, usually terminal, often born directly on the sides of emanating or mantle hyphae. Mantle becoming more compact with narrower, less papillate hyphae towards root surface, but no distinct inner mantle was noted. Uncommon in subalpine *Picea-Abies* forest. Identified on the basis of anatomical similarity to the ectomycorhizae of *Sarcodon imbricatus* (Agerer 1991a).

Thelephoraceae 1 on *Abies lasiocarpa* and (or) *Picea engelmannii*

Monopodial to monopodial pinnate. Smooth, brown. Emanating hyphae, not uncommon, 4.1-5.3 μm, clamped, brownish, cylindrical, slightly thick walled. Outer mantle

synnenchymous to pseudoparenchymous, composed of thick walled, yellow-brown, mostly rounded triangular but also rectangular or cylindrical cells 9.0-40 x 6.4-35 μm. Localized areas of mantle becoming blue-green in 5% KOH. Clavate cystidia present on outer mantle; thin walled, hyaline to pale brown, with one or two simple or clamped septa, 50-120 x 3.2-5.2 μm, up to 7.8 μm at apex. Inner mantle a tight synnenchyma of ± parallel, hyaline to yellow-brown, cylindrical hyphae (2.2 x 7.4 μm) with variable branching, often clamped. Common subalpine *Picea*-Abies forests. Identified on the basis of anatomical similarity to Thelephoraceous ectomycorrhizae described by Danielson and Pruden (1989), (Agerer 1995a, 1996), Köljalg 1992, and Goodman *et al* 1996-1997).

Thelephoraceae 2 on *Abies lasiocarpa* and *Salix barrattiana*

Monopodial to monopodial pinnate. Smooth, brown. Emanating hyphae uncommon, ± tortuous, pale to dark yellow-brown, thick walled, clamped 2.9-6.0 μm. Outer mantle a pseudoparenchyma, composed mainly of rounded triangular cells 9- 21 x 7-13 μm (often forming rosettes of 5-8 cells), but similarly sized rectangular and subglobose cells also present, as well as obtusely branched cylindrical cells 2.2-5.0 μm wide. Isolated areas of outer mantle becoming blue-green in 5% KOH. Inner mantle a tight synnenchyma of narrow, cylindrical, pale yellow-brown, acutely branched hyphae, 1.8-5.2 μm wide (mostly 3.0 μm) often forming parallel sheets, clamps common. Locally abundant at tree line. Identified on the basis of anatomical similarity to Thelephoraceous ectomycorrhizae described by Danielson and Pruden (1989), (Agerer 1995a, 1996), Köljalg 1992, and Goodman *et al* 1996-1997).

Thelephoraceae 3 on *Picea engelmannii* and *Salix barrattiana*

Monopodial to monopodial pinnate. Smooth to granulose, blackish. Emanating hyphae sometimes abundant, yellow-brown, thick walled, 4.0 -4.9 (5.9) μm, clamps at some septa Outer mantle a pseudoparenchyma composed of dark, red-brown, very thick walled, subglobose to elliptical or angular cells, 11-44 x 9-33 μm, subglobose cells often more abundant in localized areas forming mounds. Isolated areas of outer mantle becoming blue-green in 5% KOH. Inner mantle a synnenchyma of simple septate, cylindrical to ± contorted, acutely branching, thinner walled, yellow brown hyphae 1.9-8.0 μm wide. Common in the alpine zone. Identified on the basis of anatomical similarity to Thelephoraceous ectomycorrhizae described by Danielson and Pruden (1989), (Agerer 1995a, 1996), Köljalg 1992, and Goodman *et al* 1996-1997).

***Tubulicrinis* sp**. (tentative identification) on *Picea engelmannii* (figs. 4.7- 4.10)

Monopodial-pinnate to pyramidal. Smooth, yellow brown to iridescent green. Emanating hyphae uncommon, yellow-green, clamped, 2.3- 3.7 μm wide. Forming yellow to olive-green hyphal strands, ± smooth, 200 μm wide, with thicker walled contorted hyphae on outer surface. Outer mantle synnenchymous to pseudoparenchymous, of thick walled, yellow brown cylindrical, short rectangular, triangular or subglobose cells 9-40 x 4.4-17 μm. Inner mantle; a loose synnenchyma of cylindrical, obtusely branching, pale yellow, simple septate, thin walled hyphae, 2.6-5.2 μm wide. Cylindrical, amyloid lyocystidia common on surface of mycorrhizae, also on hyphal strands; hyaline to dark olive, 43-63 x

3-6 μm, with a thick outer wall over a thin walled capillary lumen which widens (up to 9.3 μm) at the apex (Figs.7-10). Locally abundant in subalpine *Picea-Abies* forest. Identified on the basis of similarity of cystidia to those on sporocarps of Tubulicrinis (see Oberwinkler 1965).

Mycorrhizae identified by direct comparison of RFLP patterns between ectomycorrhizae and sporocarps

Cortinarius calochrous (Pers.: Fr.) Fr. on *Picea engelmannii* (Fig. 4.5). Monopodial-pinnate. White, some areas cream coloured due to yellow intracellular pigments, cottony, quickly fuschia in 5% KOH, as are the basal hyphae of sporocarp. Emanating hyphae abundant clamped, hyaline to pale yellow, 2.5-3.5 μm wide, coalescing to form loose, fan-like hyphal strands. Strands without internal differentiation, also pink in KOH. Outer mantle mainly a prosenchymous reticulum of broadly branching hyphae (mantle type A of Agerer 1991b), some areas with ± parallel hyphae. Inner mantle synnenchymous, cells becoming shorter and wider, simple septate, not well differentiated from Hartig net. Microsclerotia common, white, pink in KOH, up to 1 mm in diam., subglobose to oblong, sometimes bilobed, smooth beneath surficial hyphae, covered in loose hyphal strands. Mature microsclerotia differentiated into a thin layer of appressed hyphae (similar to emanating hyphae) and a hyaline, pseudoparenchymatous interior, cells subglobose to rectangular or triangular, 6.0-19 x 3.6-16 μm. Locally abundant in subalpine *Picea-Abies* forest.

Hydnellum caeruleum (Hornem.) Karst., on *Picea engelmannii* (Figs. 4-1, 4-1b)

Monopodial-pinnate to ± coralloid, ochre-grey, dingy orange, white or pink, Cottony, some "carbonizing" (root epidermal cells becoming dark and moribund), olive in KOH (as are basal hyphae and teeth of sporocarps). Emanating hyphae abundant, simple septate, hyaline to slightly vinaceous in H_20, olivaceous in 5% KOH, 1.7 - 3.9 μm, forming abundant hyphal strands. Strands cottony, concolorous with mycorrhizae, not differentiated internally, 25-1000 μm in diam., attached to small undifferentiated, vinaceous brown hyphal mats (up to 3 x 1 cm.), which often engulf mycorrhizae. Outer mantle mainly a loose, disorganized prosenchyma of obtusely branched hyphae 2.2 - 4.1 μm., forming a reticulum in some areas. Inner mantle synnenchymous, hyphae narrower, acutely branched, often forming parallel sheets, 1.4 - 3.5 μm. Chlamydospores abundant in hyphal strands and mats, also in emanating hyphae and outer mantle; slightly thick walled, smooth, hyaline to pale brown, broadly elliptical to subglobose, mainly intercalary, 8-15 X 5.5-9.0 μm. Hyphae of strands, mats and outer mantle containing dark violet (in H_2O) crystals, which dissolve in 5% KOH giving off a blue-green diffusing pigment. Locally abundant in subalpine *Picea-Abies* forest.

Piloderma byssinum (Karst.) Jülich on *Picea engelmannii* (Fig. 4.6)

Monopodial-pinnate. White to cream, cottony. Emanating hyphae abundant (sometimes forming small hyphal pads), hyaline, simple septate, 2.2-2.8μm wide, densely ornamented with calcium oxalate crystals 1.2-3.3 x 0.2-0.5 μ m, stable in KOH. Hyphal strands composed of similar hyphae, loose, cottony, white, up to 400 μ m wide, not internally

differentiated, although outer hyphae produce a coating of mucilaginous material. Outer mantle a loose disorganized prosenchyma, hyphae again similar, but more variable in width; 1.7-4.3 μ m wide. Inner mantle a thin synnenchyma of obtusely branched or parallel, hyaline, simple septae hyphae, without calcium oxalate crystals, 1.4-4.5 μ m wide. Locally abundant in subalpine forest.

Russula integra Fr. on *Abies lasiocarpa* (Figs. 4-3, 4-4)

Unramified to monopodial-pinnate. Smooth, mantle hyaline (assuming colour of underlying root tissue). Emanating hyphae uncommon, hyaline, simple septate, 2.0-2.8 μm wide, cylindrical to tortuous. Outer mantle a thin synnenchyma of strongly anastomosing, cylindrical hyphae, 3.3-5.2 μm, and short rounded cells, 6.5-12.6 x 4.4-9.6 μm. Central mantle a synnenchyma of subglobose, bent cylindrical and epidermoidal cells, 10-18 x 2.0-7 μm, many of which are reactive (blue) in sulphovanillin. Inner mantle synnenchymous, hyphae hyaline, cylindric to tortuous, branching obtusely, 2.0-5.6 μm wide, often in parallel sheets. Locally abundant in *Abies lasiocarpa* krummholz.

Mycorrhizae identified by both comparison to published descriptions and phenetic clustering of RFLP data.

Alpine Cortinarius on *Salix barrattiana*

Monopodial pinnate. Cottony, pinkish. Hyphal strands undifferentiated, up to 350 μm wide, cottony, loose. Hyphae of strands pinkish, clamped, cylindrical, 2.2-3.7 μm wide.

Emanating hyphae abundant, similar to those forming hyphal strands. Outer mantle a disorganized prosenchyma of hyaline to pinkish, cylindrical, obtusely branched hyphae, 1.6-4.3 μm wide, often with clamp connections. Inner mantle synnenchymous; a mixture of simple septate, hyaline, cylindrical (1.5-4.4 μm) and epidermoidal (8.7-30 x 3.7-6.3) cells. Uncommon in alpine *Salix* patches.

Forest Cortinarius 1 on *Picea engelmannii*

Monopodial pinnate. Cottony, whitish with faint pinkish tones, often bent. Hyphal strands narrow, delicate, up to 200 μm wide, ± smooth, undifferentiated, faintly pinkish. Hyphae of strands cylindrical, 2.2-4.7 μm wide, clamped, hyaline, with localized intracellular yellow pigment. Emanating hyphae abundant, similar to those forming hyphal strands. Outer mantle a disorganized prosenchyma of hyaline to pinkish, cylindrical, obtusely branched hyphae, 2.4-5.0 μm wide, often with clamp connections. Inner mantle synnenchymous; a mixture of rarely clamped, hyaline to pinkish, narrow, cylindrical, acutely branched hyphae, 1.0-5.0 (6.0) μm and subglobose (4.2-14 x 3.5-11 μm) cells. Locally abundant in subalpine *Picea-Abies* forest.

Forest Cortinarius 2 on *Picea engelmannii*

Monopodial pinnate. Cottony, white, often bent. Hyphal strands undifferentiated, up to 300 μm wide, cottony, loose. Hyphae of strands hyaline, clamped, cylindrical, 2.0-5.0 μm wide. Emanating hyphae abundant, similar to those forming hyphal strands. Outer mantle a disorganized prosenchyma of hyaline, cylindrical, obtusely branched hyphae, 2.0-4.6 μm

wide, often with clamp connections. Inner mantle synnenchymous; a mixture of simple septate, hyaline, cylindrical (1.6-3.4 μm) and subglobose (4.5-10 x 3.2-6.5 μm) cells. Common in subalpine *Picea-Abies* forest.

Krummholz Cortinarius on *Picea engelmannii*

Monopodial pinnate. Cottony, white, often bent. Emanating hyphae abundant, hyaline, clamped, cylindrical, 2.3-5.4 μm wide. Outer mantle a disorganized prosenchyma of hyaline, cylindrical, obtusely to acutely branched hyphae, 2.4-5.4 μm wide (usually 2.8 μm) often with clamp connections. Inner mantle a disorganized synnenchyma of simple septate, hyaline, cylindrical to tortuous hyphae, 1.6-3.5 μm wide. Common in krummholz islands of *Picea* and *Abies* at tree line.

Laccaria sp. on *Picea engelmannii* and (or) *Abies lasiocarpa*

Monopodial to monopodial pinnate. ± smooth, dull orange-brown (concolorous with sporocarps of *Laccaria proxima*). Emanating hyphae abundant, 1.5-2.9 μm wide, clamped, hyaline, cylindrical to slightly tortuous. Outer mantle a loose prosenchymous reticulum formed by obtusely branched hyphae, 1.8-4.5 μm wide (usually 2.5μm) mostly simple septate but clamps present, often with short side branches (elbows), similar to the mantle of *Laccaria proxima* (Ingelby *et al.* 1990). Inner mantle a synnenchyma of cylindrical to tortuous, acutely branched, hyaline, simple septate hyphae, 1.8-3.5 μm wide, in ± parallel orientation. Common in subalpine *Picea-Abies* forests.

Alpine Inocybe on *Dryas octopetala*

Monopodial to monopodial pinnate. Smooth, tawny to yellow olivaceous. Emanating hyphae not uncommon, narrow, 2.6-3.1 μm, clamped, hyaline, cylindrical to tortuous. Outer mantle a prosenchyma of repeatedly branched simple septate hyphae, 2.0 - 6.0 μm often wider at branch points (up to 9.1 μm), branching obtuse, forming 2, 3 and 4 way intersections, similar to the mantle of *Inocybe lacera* (Ingelby *et al.* 1990). Septa and some cellular contents staining bright blue in lactophenol cotton blue. Inner mantle a synnenchyma of acutely branched hyaline, simple septate, cylindrical to subglobose or epidermoidal cells 5.9-17 x 1.9-3.5 μm. Locally abundant in mats of alpine *Dryas octopetala*.

Unidentified mycorrhizae

Alpine Ascomycete 1 on *Salix barrattiana*

Monopodial. Granular, dark red-brown to blackish. Emanating hyphae dark brown, very thick walled (up to 2.6 μm) simple septate, septa often only 20 μm apart, often strongly papillate 6.5-10.4 μm wide at base, tapering downwards to 4.0 μm away from mantle. Outer mantle pseudoparenchymatous, composed of dark yellow-brown, thick walled globose to elliptical cells, 5.6 -23 X 5.2-26 μm in diam. Inner mantle similar; cells becoming thinner walled, more rectangular and pale yellow.

Alpine Ascomycete 2 on *Dryas octopetala* and *Salix barrattiana*

Monopodial. Smooth, brown. Emanating hyphae not uncommon, hyaline to pale yellow,

simple septate 2.4-4.5 (5.4). Outer mantle a pseudoparenchyma of pale brown, interlocking epidermoidal cells ("jig-saw"), 8.0-45 x 2-12 μm (Mantle type M of Agerer 1991ba). Inner mantle similar but less regular; cells pale yellow, epidermoidal shape less pronounced, some cylindrical cells (2.7-4.4 μm) also present. Common in mats of alpine *Dryas octopetala* and patches of *Salix barrattiana*

Forest Basidiomycete 1 on *Picea engelmannii* and (or) *Abies lasiocarpa*

Monopodial pinnate, ± smooth, orange brown, strands up to 175 μm wide, orange brown ± flattened. Hyphae of strands clamped at every septum, yellow-orange, ± thick walled or hyaline and thin walled, 4.6-5.1 μm wide. Emanating hyphae 2.4-5.4 μm wide, clamped. Outermost mantle a prosenchyma of cylindrical, hyaline, clamped hyphae forming a regular and symmetric reticulum over a pseudoparenchymous inner mantle (similar to mantle type P of Agerer 1991b). Inner mantle cells hyaline simple septate, subglobose, triangular, rectangular or obtusely branched cylindrical cells, 8.0-27 x 4.5-9.0 μm.

Locally abundant in subalpine *Picea-Abies* forest.

Forest Basidiomycete 2 on *Picea engelmannii*

Monopodial pinnate. Mantle web- like, whitish to pale cream. Emanating hyphae abundant, cylindrical, hyaline to very pale yellow, clamped, 2.5-8.7 μm wide. Outer mantle a disorganized prosenchyma of hyaline, cylindrical, obtusely branched, simple septate hyphae, 2.4-5.4 μm wide (usually 2.8 μm). Mantle becoming more synnenchymous towards root surface but no distinct inner mantle was noted. Uncommon in subalpine

Picea -Abies forest.

Discussion

Some of the mycorrhizae collected from montane Alberta are formed by essentially ubiquitous fungi which colonize a wide variety of hosts in diverse habitats; *Cenococcum geophilum*, *Amphinema byssoides* and *Tomentella* spp. are components of most ectotrophic communities and can be found throughout the temperate northern hemisphere (Trappe 1964, Jülich and Stalpers 1980). Many of the mycorrhizae collected, however, are formed by fungi with distributions restricted to northern and montane habitats and associations with *Abies, Picea, Dryas* or *Salix*.

Russula integra, an *Abies* associate known mainly from montane and subalpine coniferous forests in Europe and North America (Chapter Two of this thesis, Kernaghan and Currah in press) is an example of one these more restricted taxa. The mycorrhizae are anatomically similar to those of another *Abies* associate, *Russula silvicola* (Kernaghan *et al.* 1997), but differ in having epidermoidal, rather than polygonal sulphovanillin reactive cells in the mantle.

Cortinarius calochrous subsp. *coniferarum* also has an essentially northern and montane distribution, known from European boreal and montane coniferous forests and the mountains of western North America (Kernaghan and Currah in press). The microsclerotia production by *C. calochrous* is the first example of its kind in the genus *Cortinarius*, but they have been described by some authors on *Hebeloma crustuliniforme* (Bull. ex St. Amans) Qúel., another member of the Cortinariaceae (Zak 1973, Voiry

1981). Microsclerotia production in ectomycorrhizal basidiomycetes is most commonly seen in members of the Boletales e.g. *Paxillus involutus* (Batsch) Fr. (Ingelby 1990, Gronbach 1988), *Gyrodon lividus* (Bull.: Fr.) Sacc. (Agerer *et al.* 1993) and *Leccinum* sp. (Ingelby 1990). The fuchsia KOH reaction is very distinctive and can also be seen in the basal hyphae of the sporocarp, the pileus and cortina. This reaction is common in sporocarps of *Cortinarius* subgen. *Phlegmacium* sec. *Calochroi* as well as in other Phlegmaciums. (Moser 1983). Similar KOH reactions have been noted in the ectomycorrhizae of *Dermocybe cinnabarina* (Fr.) Wünsche (Brand 1991, as *Cortinarius cinnabarinus* Fr.) and *Cortinarius armillatus* (Fr.) Fr. (Thoen 1979). In both cases the sporocarps also exhibit the same reactions.

The other *Cortinarius* mycorrhizae described, "Forest Cortinarius" 1-2, "Krummholz Cortinarius" and "Alpine Cortinarius" share general morphological and anatomical features with each other, with *C. calochrous* and with other *Cortinarius* mycorrhizae described in the literature (Agerer 1987a, 1988 Groenbach 1988, Cuvelier 1990, Brand 1991). These include thin walled, unornamented hyphae, a disorganized prosenchymous outer mantle, loose, undifferentiated hyphal strands, and clamped hyphae in strands and outer mantle. Differences among the *Cortinarius* mycorrhizae collected include pigmentation, abundance of hyphal strands and, in *C. calochrous*, a fuchsia KOH reaction and microsclerotia production.

Most *Cortinarius* mycorrhizae have few distinguishing characteristics, however, and could be confused with the mycorrhizae of other genera, most notably clamped species of *Tricholoma*. To date, descriptions of *Tricholoma* mycorrhizae (Uhl 1988, Agerer 1987b,

Brand 1991, Waller and Agerer 1993) do not include clamp connections in the outer mantle, as seen in *Cortinarius* mycorrhizae. This is corroborated by our phenetic analysis (Fig. 4.12), in which the mycorrhizae suspected of being Cortinariaceous (with clamps in outer mantle) clustered within the *Telamonia* or *Phlegmacium/Myxacium* clades (Fig. 4.12), and the mycorrhizae with simple septate hyphae in the outer mantle clustered with the Tricholomataceae.

A possible mycobiont of the pink *Telamonia*, "Alpine Cortinarius" on *Salix* is *Cortinarius inops* Favre *sensu* Möller, a *Salix*-associated *Telamonia* with pink basal hyphae, collected in the alpine zone of both sites. It seems unrealistic, however, to hypothesize on the possible species of *Cortinarius* responsible in the other two cases, given that sporocarps of 29 Cortinarii were collected (Chapter Two of this thesis, Kernaghan and Currah in press).

Hydnellum caeruleum is also a northern conifer associate, although it is found with broadleaved trees when occurring at lower latitudes (Chapter Two of this thesis, Kernaghan and Currah in press). The mycorrhizae are similar in general morphology and anatomy to those formed by *Hydnellum peckii* (Agerer 1993); both are carbonizing, form abundant rhizomorphs and distinctive chlamydospores. The main differences include the silvery-white colour, lack of olive reaction in KOH, and the yellow, spherical, warted chlamydospores in *H. peckii*. The chlamydospores of *H. caeruleum* resemble immature *H. peckii* chlamydospores but are single walled, spherical to ellipsoid and are smooth rather than warted. Chlamydospores found in the mantles of other Thelephoraceous mycorrhizae are described by Agerer (1991b). The dark violet, intra-hyphal crystals releasing blue-

green pigment in KOH are also found in sporocarps of *H. caeruleum* as well as other species of *Hydnellum* and *Sarcodon* (Harrison 1964) and are thought to contain thelephoric acid (Agerer 1993). They are also described from the mycorrhizae of *H. peckii* (after storage in lactic acid)(Agerer 1993) and the fresh mycorrhizae of *Albatrellus ovinus* (Agerer *et al.* 1996a).

Piloderma byssinum is found throughout the north temperate hemisphere in coniferous and mixed forests (Eriksson *et al.* 1981). It is more common in poorer, less acidic forests than the well known *P. fallax* (Libert) Stalpers (Eriksson *et al.* 1981, as *P. bicolor*), described by Brand (1991)(as *P. croceum* Erikss. and Hjortst.) and Goodman *et al.* (1996-1997). The sporocarps and mycorrhizae are anatomically similar in both species but *P. byssinum* lacks the distinctive bright yellow pigment present in the hyphal strands of *P. fallax* (Larsen *et al.* 1996). Froideux (1975) describes the mycorrhizae of *P. byssinum* on *Pseudotsuga menziesii*. It is unclear however, whether Froideux collected *P. byssinum* or *P. fallax*. Because he describes the mycorrhizae and sporocarps as having bright yellow pigment, it is likely that he had collected the mycorrhizae of the more common *P. fallax*.

Both *P. fallax* and *P. byssinum* can produce large quantities of needle-shaped calcium oxalate crystals. Crystal formation is dependent on the calcium concentration in the soil (Graustein et al. 1977), however, and mycorrhizae of *Piloderma* are therefore likely to show significant variation in this character. The lack of crystal formation on the inner mantle hyphae of *P. byssinum* may be due to a lack of direct contact with calcium in the soil.

Although no associated sporocarps were collected, the ectomycorrhizae of *Tubulicrinis* are tentatively identified based on their distinctive amyloid lyocystidia. Lyocystidia occur in other corticioid genera including *Litschauerella* and *Tubulicium*, but only in *Tubulicrinis* are they cylindrical, amyloid and unornamented (Oberwinkler 1965, Hjortstam *et al.* 1987, 1988). *Tubulicrinis accedens* (Bourd. and Galz.) Donk is a possible candidate for the mycobiont in the described symbiosis; it has similar cystidia, fugaceous sporocarps and is associated with *Picea* (Hjortstam *et al.* 1988). *Tubulicrinalis* has not been reported as an ectomycorrhizal genus, but given the diversity and ubiquitous nature of corticioid taxa, it is not surprising to find a new symbiosis involving a corticioid fungus.

The mycorrhizae identified as being formed by *Laccaria* from the subalpine forest did not share the RFLP pattern of the only *Laccaria* species collected on the sites, *Laccaria montana* Sing., a circumpolar and alpine species. The RFLP patterns were similar however, and the two taxa clustered within the same clade (Fig. 4.12). The mycorrhizae are anatomically similar to those formed by *L. proxima* (Boudier) Patouillard (Ingelby 1990) which, along with *L. laccata* (Scop.: Fr) Cooke var. *pallidifolia* (Peck.) Peck and *L bicolor* (Maire) Orton, is commonly associated with conifers in the region (Mueller 1992).

The mycorrhizae identified as being formed by *Inocybe* from the alpine zone produced an RFLP pattern which did not match the pattern of any of the *Inocybe* species analyzed (Table 4.2). Phenetic clustering analysis of RFLP data placed the mycobiont within *Inocybe* (Fig. 4.12), however, a designation also supported by anatomical characters. The compact reticulate mantle formed by simple septate hyphae producing thin, clamped emanating hyphae, and the reaction to lactophenol-cotton blue, appear to be a strong

combination of characteristics for the identification of *Inocybe* mycorrhizae (see Ingelby 1990, Cripps and Miller 1996, Agerer *et al.* 1996b). As with *Cortinarius*, it is difficult to deduce the species involved due to the large number of possibilities.

The mycorrhizae formed by a species of *Sarcodon* are characterized by distinctive nodulose chlamydospores, and the wide, thick-walled, papillate hyphae as described by Agerer (1991a) on the mycorrhizae of *Sarcodon imbricatus* (L.: Fr.) Karst. These mycorrhizae differ from those of *S. imbricatus* by the yellow pigment, lack of clamp connections, and by the lack the abundant hyphal strands described by Agerer (1991a), but the latter may be due to microsite variability which could not be tested because only one collection was made. The RFLP pattern of the *Sarcodon* mycorrhizae did not match that of the most common species of *Sarcodon* fruiting on the site, *S. cf. versipellis* (Fr.) Quel., although they both possess a relatively large ITS region, 744 bp and 770 bp respectively. *S. scabrosus* (Fr.) Karst. is a possible candidate for the mycobiont because it does occur in the subalpine *Picea/Abies* forest (Kernaghan and Currah in press) but was not found on the site. The morphology of *S. scabrosum* mycorrhizae are briefly described by Ogawa (1985), but no anatomical details, such as presence of chlamydospores, are given.

The mycorrhizae referred to as "Thelephoraceae" 1-3, were identified on the basis of their dark, pseudoparenchymatous mantles, dark, thick-walled, clamped emanating hyphae, cyanesence in KOH, and in the case of Thelephoraceae 1, distinctive cystidia. Similar characters are noted in other mycorrhizae identified as *Tomentella* or *"Tomentella* like" (Danielson and Pruden 1989, Agerer 1995a, 1996, Köljalg 1992, Goodman *et al.*). Thelephoraceae 3 is very similar to the unidentified mycorrhizae of *Picea* "Piceirhiza

nigra" described by Groenbach (1988). The confirmation of thelephoric acid in "Piceirhiza nigra" by HPLC indicates a thelephoraceous mycobiont, possibly a species of *Tomentella* (Agerer *et al.* 1995a).

The cyanesence of hyphae in KOH seen in "Thelephoraceae" 1-3 is likely a pH mediated change in thelephoric acid (Burdsall and Setliff 1974, Gill and Steglich 1987, Agerer 1995a), and has been noted in sporocarps of the Bankeraceae (discussed above in *Hydnellum*) and the Thelephoraceae (Stalpers 1993, Koljalg 1996), as well as the mycorrhizae of *Tomentella radiosa* (Bourdot and Galzin) Rick (as *Tomentella albomarginata* (Bourdot and Galzin) M.P. Christ.)(Agerer 1996).

The Thelephoraceae *sensu* Stalpers (1993), includes nine genera, four of which (*Thelephora, Tomentella, Tomentellastrum* and *Pseudotomentella*) exhibit cyanesence of hyphae in KOH. Of these, *Tomentellastrum* and *Pseudotomentella* have simple septate hyphae, leaving species of *Thelephora* (including resupinate forms) and (or) *Tomentella* as possible mycobionts of our "Thelephoraceae" mycorrhizae.

Sporocarps of *Tomentella ellisii* (Sacc.) Jülich and Stalpers, *T. sublilacina* Ellis and Holway) Wakef. and *Thelephora caryophyllea* (Schaeffer: Fr.) Fr. were collected on the sites (Kernaghan and Currah in press). RFLP data was obtained for *T. caryophyllea* and "Thelephoraceae" 3, as well as *Thelephora terrestris* Ehrh.: Fr. and *Tomentella umbrinospora* Larsen, from other habitats (Table 4.2). ITS region sizes were similar among *Thelephora* spp. and *Tomentella umbrinospora* (685 bp ± 2.5 %) but larger in "Thelephoraceae" 3 (738 bp). This does not rule out the possibility of the mycobiont in "Thelephoraceae" 3 belonging to the Thelephoraceae, however, given that other genera

i.e. Inocybe and *Cortinarius*, exhibit two ITS size classes which essentially correspond to subgenera (Table 4.2).

In general, very little is known about the level of genetic variation in the Thelephoraceae, in part due to the difficulty involved in the extraction and amplification of DNA from its woody or resupinate sporocarps. Gardes and Bruns (1996) amplified an ITS region from *T. sublilacina* mycorrhizae of at least 851 bp (using a primer combination which gives slightly larger amplicons than those used here) and Kårén and Nylund (1997) found RFLP variation among sporocarps of *Thelepora terrestris* collected at different locations.

"Alpine Ascomycete" 1, specific to the roots of *Salix* in the alpine zone, is anatomically very similar to the ectomycorrhizae of *Genea hispidula* Berk. *et* Br. described on *Fagus sylvatica* by Brand (1991). We hesitate to refer to these mycorrhizae as *Genea* however, as sporocarps have never been reported from Western Canada and *Genea* has not been reported in association with *Salix* (Trappe 1971, Maia *et al.* 1996).

"Alpine Ascomycete" 2 was common on the roots of *Salix* and *Dryas* in the alpine zone. Of known ectomycorrhizae, it appears most similar to those of *Tuber* spp., especially with respect to the anatomy of the outer mantle (Voiry 1981, Blaschke 1987, Rauscher et al. 1995) but lacks the characteristic long, pointed cystida usually found on *Tuber* mycorrhizae.

The mycobionts involved in "Alpine Ascomycete" 1-2 are possibly hypogeous or sub-hypogeous members of the Pezizales, because of their anatomical similarities to *Genea* and *Tuber* and the high proportion of ectomycorrhizal ascomycetes belonging to the Pezizales

(Trappe 1971, Maia *et al.* 1996). *Hydnotria cubispora* (Bessey and B.E. Thompson) Gilkey was collected in the nearby subalpine *Picea-Abies* forest (Chapter Two of this thesis, Kernaghan and Currah in press) and *Geopora arenosa* (Fuckel) Ahmad, has been collected with alpine *Salix* in the region (S. Abbott, pers. comm.). DNA analysis was not carried out on "Alpine Ascomycete" 1-2 because of the lack of ascomycetous sporocarps collections on the sites.

"Forest Basidiomycete 1" has an outer mantle pattern unlike any described previously. Pseudoparenchymatous mantles with an overlying reticulum of simple septate hyphae have been described from Russulaceous mycorrhizae (Agerer 1995b), but the presence of clamp connections clearly precludes a Russulaceous mycobiont. Because "Forest Basidiomycete 1" clusters with *Hygrophorus chrysodon* (Fr.) Fr. and *Catathelasma imperiale* (Fr.) Sing. in the phenetic analysis, and has a smooth, clamped mantle, it is possibly a symbiosis formed by a clamped Tricholomataceous mycobiont such as a species of *Hygrophorus.*

"Forest Basidiomycete 2" has anatomical similarities to the mycorrhizae of *Tricholoma* (*i.e.* hyaline hyphae forming disorganized mantles and hyphal strands), and clustered with *Tricholoma* and *Laccaria* in the phenetic analysis. The only *Tricholoma* sporocarps possessing clamp connections collected on the site however, were those of *T. saponaceum* Staude, which gave a different RFLP profile. "Forest Basidiomycete 2" is therefore most likely a clamped member of the Tricholomataceae, perhaps a *Tricholoma* for which the sporocarp was not collected.

Phenetic clustering of RFLP data has been used by Baldwin and Egger (1994) and Kårén and Nylund (1997) for the identification of unknown ectomycorrhizae. In both cases

the phenograms suffered from homoplasy, with unrelated taxa clustering together. We attempted to minimize this effect by limiting each phenogram to taxa from one or two families. Clustering of the RFLP data from Kernaghan *et al.* (1997)(Chapter Three of this thesis) clearly demonstrated the ability of this approach to separate closely related genera (Fig. 4.11). Similar analysis of data from members of the Cortinariaceae and Tricholomataceae separated the two families and, in the Cortinariaceae, separated genera. Delineation of subgenera within *Cortinarius* and *Inocybe* was also evident to some degree (Fig. 4.12). As expected, unidentified mycorrhizae fell into clades which corroborated previous morphological identifications and, for the mycorrhizae of *Cortinarius*, provided evidence as to the possible subgenus.

The approach used involves detailed anatomical descriptions in combination with molecular analyses for the identification of ectomycorrhizae. The information provided should facilitate subsequent re-identification by other researchers using either anatomical or biochemical characters.

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Table 4.1. Collecting locations and accession numbers of sporocarps used for RFLP analysis.

Fungus	Location	Accession #
Amphinema byssoides	Mt. Tripoli	ALTA 10351
Catathelasma imperiale	Mt. Rae	ALTA 10367
Cortinarius cf. biformis	Mt. Rae	ALTA 10401
C. brunneus	Mt. Tripoli	ALTA 10146
C. calochrous	Mt. Rae	ALTA 10185
C. delibutus	Mt. Rae	ALTA 10199
C. evernius	Mt. Rae	ALTA 10154
C. favrei	Mt. Tripoli	ALTA 10209
C. glaucopus	Mt. Tripoli	ALTA 10183
C. hinnuleus	Mt. Rae	ALTA 10158
C. multiformis	Mt. Tripoli	ALTA 10192
C. muscigenus	Mt. Rae	ALTA 10212
C. percomis	Mt. Rae	ALTA 10196
C. triformis	Mt. Tripoli	ALTA 10173
C. uraceus	Mt. Tripoli	ALTA 10177
Dermocybe crocea	Mt. Rae	ALTA 10229
Hebeloma crustiliniforme	Robb AB	ALTA 10400
H. insigne	Mt. Tripoli	ALTA 10284
Hydnellum caeruleum	Mt. Rae	ALTA 10272
Hygrophorus chrysodon	Mt. Tripoli	ALTA 10330
H. korhonenii	Mt. Rae	ALTA 10337
H. pudorinus	Mt. Tripoli	ALTA 10343
Hysterangium separabile	Mt. Rae	ALTA 10198

Inocybe dulcamara	Mt. Tripoli	ALTA 10311
I. geophylla	Jasper Nat. Park AB	ALTA 10402
I. lacera	Mt. Tripoli	ALTA 10320
I. lanuginosa	Jasper Nat. Park AB	ALTA 10403
I. rimosa	Mt. Tripoli	ALTA 10323
I. whitei	Mt. Tripoli	ALTA 10325
Laccaria montana	Mt. Tripoli	ALTA 10384
Piloderma byssinum	Bragg Creek AB	ALTA 10143
P. fallax	Brown-lowery Prov. Park	ALTA 10144
Russula integra	Mt. Tripoli	ALTA 9880
Thaxterogaster pingue	Mt. Rae	ALTA 10349
Thelephora caryophyllea	Mt. Tripoli	ALTA 10392
Thelephora terrestris	Edmonton AB	ALTA 10399
Tomentella umbrinospora	Edmonton AB	ALTA 10398
Tricholoma saponaceum	Mt. Rae	ALTA 10295
Tricholoma virgatum	Mt. Rae	ALTA 10305
Tuber rufum	Notikewin Park AB	ALTA 9086
Sarcodon cf. versipellis	Mt. Tripoli	ALTA 10395

Table 4.2. Restriction fragment sizes and non-digested ITS region sizes of ectomycorrhizae and sporocarps. Values in bold are common between sporocarps and mycorrhizae. Values not in bold are from either sporocarps or mycorrhizae. Names in italics refer to sporocarps, non-italicized names refer to mycorrhizae.

	Enzyme				
Taxon	*Alu*I	*Hha*I	*Hinf*I	*Rsa*I	ITS Region
Amphinema byssoides	**491** **120**	**343** **154** **122**	**322** **296**	**614**	**614**
Catathelasma imperiale	435 126 98	382 280	355 301	662	650
Cortinarius biformis	488 115	738	416 171 130	404 321	738
Cortinarius brunneus	474 104	350 292	320 299	642	642
Cortinarius calochrous	**512** **88**	**415** **286**	**400** **305**	**715**	**715**
Cortinarius delibutis	452 115	632	336 279	304	632
Cortinarius evernius	457 104	350 292	302 283	620	632
Cortinarius favrei	537 113	416 304	390 330	719	719
Cortinarius galopus	541 113	416 304	393 330	725	715
Cortinarius hinnuleus	461 103	350 292	302 289	626	632

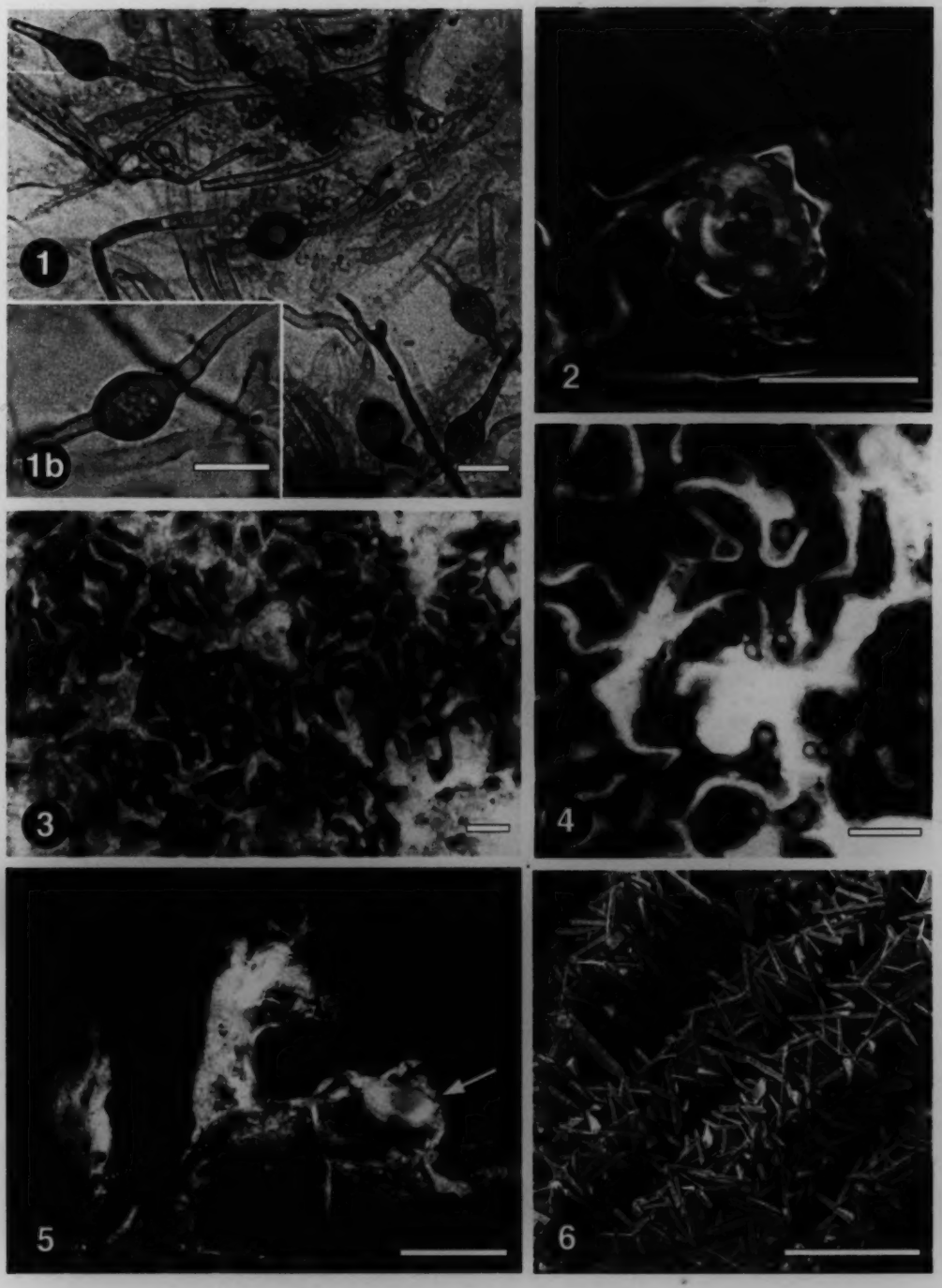
Cortinarius multiformis	478	310	306	514	715
	178	246	188	201	
Cortinarius muscigenus	541	416	390	719	719
	112	304	330		
Cortinarius percomis	471	413	393	719	713
	115	304	330		
Cortinarius triformis	466	632	342	324	632
	115		279	306	
Cortinarius uraceus	470	347	316	632	632
	119	286	299		
Dermocybe crocea	624	738	410	383	711
	115		295	320	
Hebeloma crustiliniforme	322	402	392	738	738
	193	202	259		
		118			
Hebeloma insigne	no data	no data	no data	no data	731
Hydnellum caeruleum	**457**	**347**	**365**	**754**	**738**
	176	**285**	**168**		
	127	**127**	**133**		
			86		
Hygrophorus chrysodon	263	368	345	657	658
	215	301	321		
	107				
Hygrophorus pudorinus	355	348	326	636	636
	102	297	317		
Hygrophorus korhonenii	368	347	315	625	633
	104	292			
Hysterangium separabile	284	376	343	682	682
	245	308	206		
	157		132		
Inocybe dulcamara	550	330	327	435	800
	252	315	220	349	
			189		

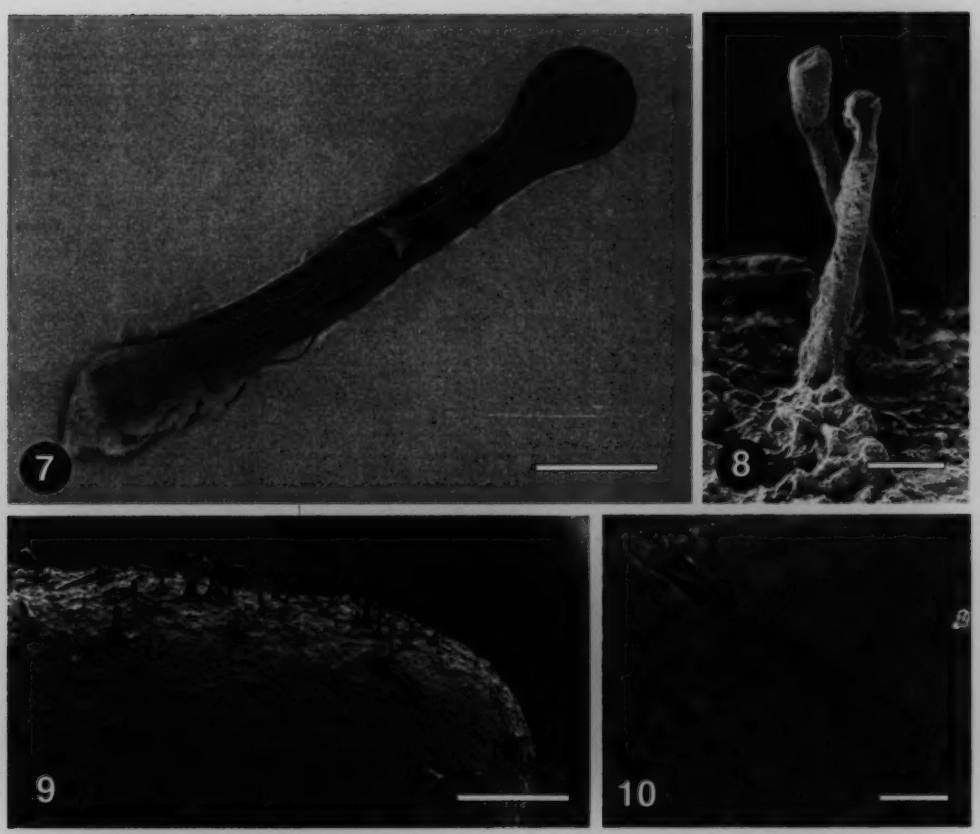
Inocybe geophylla	593	212	313	430	738
			277	286	
Inocybe lacera	537	441	201	430	765
	224	333	170	327	
			143		
			128		
Inocybe lanuginosa	499	409	384	504	705
	204	307	324	202	
Inocybe rimosa	503	413	380	547	699
	185	298	309	151	
Inocybe whitei	581	283	304	315	705
	136	235	285	293	
		191	129	110	
Laccaria montana	392	429	392	535	738
	232	311	322	199	
	111				
Piloderma byssinum	**361**	**371**	**357**	**663**	**744**
	201	**130**	**151**		
Piloderma fallax	no	no	no	663	740
	data	data	data		
Russula integra	**441**	**355**	**350**	**680**	**720**
	218	**360**	**340**		
Sarcodon cf. versipellis	389	377	372	523	770
	210	270	248	184	
	147	130	137		
Thaxterogaster pingue	528	416	393	719	713
		304	330		
Thelephora caryophyllea	256	377	350	480	679
	124	286	325	204	
	108				
Thelephora terrestris	597	377	346	231	680
	80	309	231	114	
			110		

Tomentella umbrinospora	457 198	390 169 151	350	699	696
Tricholoma saponaceum	496 96 86	302 215	390 318	620 102	720
Tricholoma virgatum	298 260 133	431 310	406 330	731	730
Tuber rufum	454 283	523 308	297 213 123 78	712 123	828
Tubulicrinis sp.	512 152	362 255 130	365	653	738
Forest Basidiomycete 1	422 121	660	336	370 301	660
Forest Basidiomycete 2	510 190	349 252 124	410 320	738	738
Alpine Cortinarius	483 117	344 172 126	319	328 215	632
Forest Cortinarius 1	472 97	336	368	640	640
Forest Cortinarius 2	504 222	352 250	372	653	719
Krummholz Cortinarius	466 117	346 295	315	642	635
Alpine Inocybe	668	365 201	319	678	678

Laccaria sp.	329 196 141	415 231	399 261	730	730
Thelephoraceae 3	329 198 141	415 214	395 264	732	738
Sarcodon sp.	512 224	345 245 134	365 275	663	744

Figs. 4.1-6. Anatomical and morphological features of ectomycorrhizae. Figs. 1 and 1b. Chlamydospores in mantle of *Hydnellum caeruleum* on *Picea engelmannii.* Scale bars = 10μm. Fig. 2. Chlamydospore of *Sarcodon* sp. on *Picea engelmannii* in Ponceau -S. Scale Bar = 10 m. Figs. 3-4. Sulphovanillin reactive epidermoidal cells in mantle of *Russula integra* on *Abies lasiocarpa*. Scale bars = 10 μm. Fig. 5. Mycorrhizal system of *Cortinarius calochrous* on *Picea engelmannii.* Note microsclerotium (arrow). Scale bar = 1 mm. Fig 6. Mantle hyphae of *Piloderma byssinum* on *Picea engelmannii* ornamented with calcium oxalate crystals. Scale bar = 5 μm.


1
1b
2
3
4
5
6

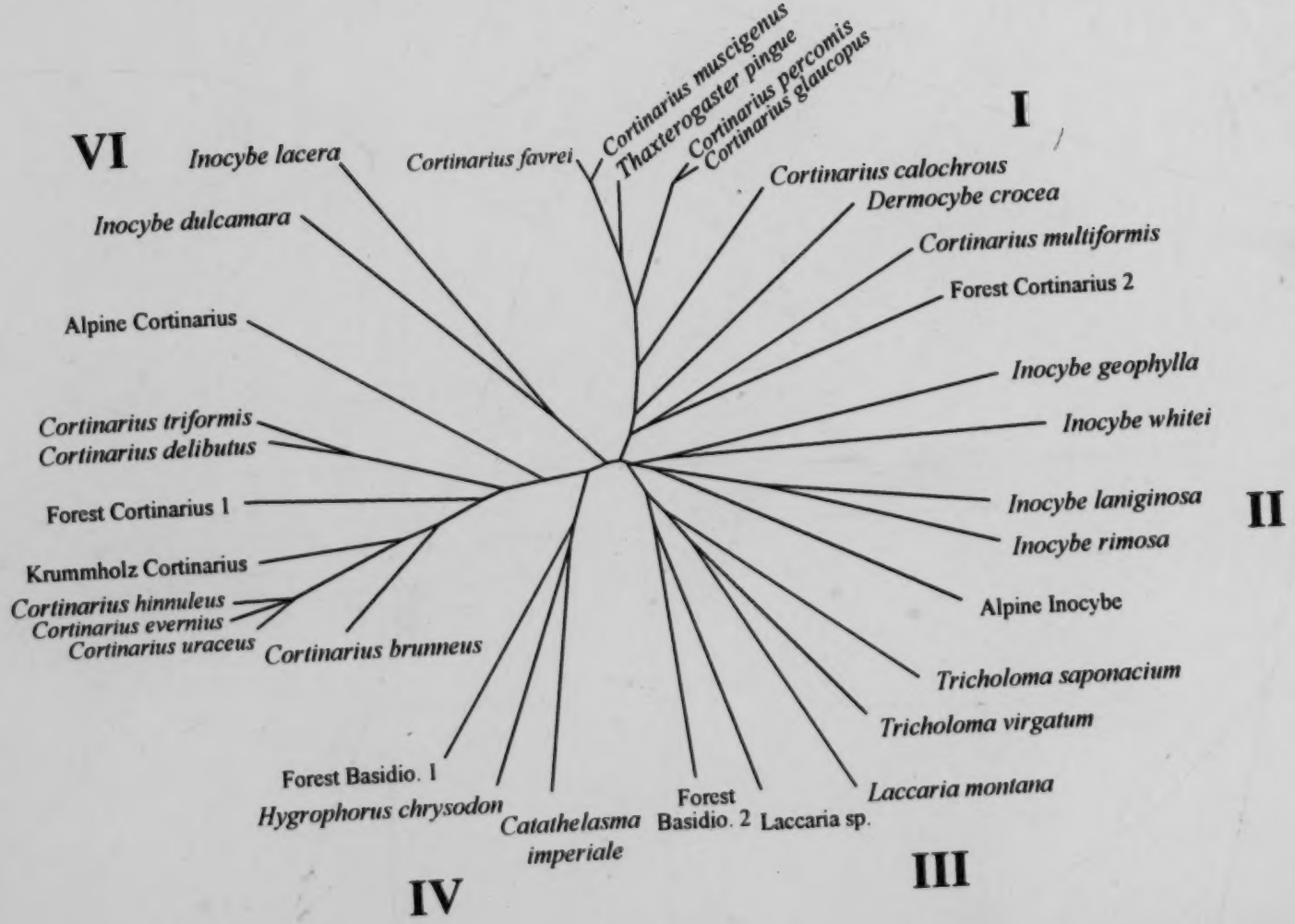


Figs. 4.7-10. Amyloid lyocystidia of *Tubulicrinis* sp. (tentative identification) on *Picea engelmannii*. Figs 7 and 10. Single lyocystidia in Melzer's reagent. Scale bars = 10μm. Figs. 8 and 9. Lyocystidia on mantle surface. Scale bar = 10μm in Fig. 8 and 100μm in Fig 9.

Figure 4.11 Neighbor joining tree of Russulaceae analyzed. Data from Kernaghan *et al.* 1997. Bold roman numerals indicate separate clades.

III
Tuber rufum
Macowanites americana
Hygrophorus pudorinus
Russula nigricans
Lactarius uvidus
Russula xerampelina
Lactarius pubescens
Russula silvicola
II
I
Lactarius luculentus
Russula brevipes
Russula fragilis
Lactarius deliciosus
Russula integra
Lactarius caespitosus
Lactarius alnicola
Russula torulosa

Figure 4.12. Neighbor joining of Cortinariaceae, Tricholomataceae and unidentified mycorrhizae. Bold roman numerals indicate separate clades.


I
II
III
IV
V
VI
Cortinarius muscigenus
Thaxterogaster pingue
Cortinarius percomis
Cortinarius glaucopus
Cortinarius favrei
Inocybe lacera
Inocybe dulcamara
Alpine Cortinarius
Cortinarius triformis
Cortinarius delibutus
Forest Cortinarius 1
Krummholz Cortinarius
Cortinarius hinnuleus
Cortinarius evernius
Cortinarius uraceus
Cortinarius brunneus
Cortinarius calochrous
Dermocybe crocea
Cortinarius multiformis
Forest Cortinarius 2
Inocybe geophylla
Inocybe whitei
Inocybe laniginosa
Inocybe rimosa
Alpine Inocybe
Tricholoma saponacium
Tricholoma virgatum
Laccaria montana
Laccaria sp.
Forest Basidio. 2
Catathelasma imperiale
Hygrophorus chrysodon
Forest Basidio. 1

Chapter Five

Community structure of ectomycorrhizal fungi across an alpine/subalpine ecotone[1]

Introduction

Ecotones, or zones of transition between habitats (or biomes), are often areas of enriched species diversity due to the co-occurrence of species from adjacent habitats (Hansen *et al.* 1992, Risser 1995). Leopold (1933) coined the term "edge effect" for this phenomenon at abrupt ecotones, and it has since been demonstrated in a wide range of organisms at various scales, including vascular plants (Jones and Peterson 1970, Scheiner and Istock 1994), small mammals (Sekgororoane and Dilworth 1995), insects (Downie *et al.* 1996) and soil fauna (Rusek 1992).

At the alpine/subalpine ecotone of the Canadian Rockies, the *Picea-Abies* forest merges with the low growing shrubs dominating the alpine zone, resulting in a mosaic of alpine and subalpine vegetation. Many of the plant genera in this ecotone are obligately ectomycorrhizal and, as such, depend on mutualistic fungi to increase their water and nutrient absorbing capabilities. Moreover, ectomycorrhizal fungi are often host specific, at the level of host plant genera (Molina *et al.* 1992). We might therefore expect the distribution of ectomycorrhizal fungi to mirror patterns, such as high diversity at the ecotone, seen in ectotrophic vegetation.

The altitude at which montane ecotones occur is determined mainly by climate,

[1]Being prepared for submission to Ecography.

although other influences, such as edaphic factors, also play a role (Hansen-Bristow *et al.* 1988, Kupfer and Cairns 1996). Because ectomycorrhizal fungi are thought to increase the elevational range of host plants by increasing their ability to tolerate harsh conditions (Moser 1980), the position of tree line should also be partially determined by the structure of the ectomycorrhizal community.

Community analyses of ectomycorrhizal fungi have only recently been undertaken. Several studies relate macromycete sporocarp distribution to host plant composition and (or) edaphic factors (Hansen 1988, Tyler 1989, Rücker *et al.* 1990, Rühling and Tyler 1990, Nantel and Neumann 1992, Såstad 1995). The number and distribution of sporocarps, however, may not be reliable indicators of the distribution of fungal thalli in the soil environment (Gardes and Bruns 1996). Ectomycorrhizae, however, are less ephemeral than sporocarps and exhibit a higher correlation between abundance and importance in ecosystem functioning. Relatively few studies, however have attempted to relate the abundance of ectomycorrhizae to environmental variables (Agerer 1985, Cázares 1992, Rao *et al.* 1997, Väre *et al.* 1997).

With respect to ectomycorrhizal fungi and ecotones, Moser (1980) studied sporocarp distribution across the montane ecotone, or "Kampfzone", in the Alps, and Hagerman (1997) quantified ectomycorrhizae across the anthropogenic ecotones formed by timber harvest, but analysis of ectomycorrhizae across a natural ecotone has not been performed. With this in mind, the purpose of this study was to relate the distribution, richness and diversity of ectomycorrhizal fungi to that of ectotrophic host plants and edaphic factors across elevational tree line. The main objective was to determine if the

diversity of ectomycorrhizal fungi was higher at the ecotone than in the adjacent habitats. A second objective was to assess differences in fungal species composition between the subalpine forest and the alpine zone in order to infer the potential of the alpine mycorrhizal community to enhance conifer establishment above the existing tree line.

Canonical correspondence analysis (CCA)(Ter Braak 1986) was employed in order to visualize relationships between species of ectomycorrhizal fungi, ectotrophic host plant abundance and edaphic factors. In CCA the ordination axes are constrained to be linear combinations of environmental variables, thus displaying the relationships between environmental variables and variation in species composition. Correspondence analysis is widely used to describe plant communities (Ludwig and Rynolds 1988) but has only recently been applied to their associated mycorrhizal fungi (Nantel and Neumann 1992, Gulden *et al.* 1992, Såstad 1995).

Materials and methods

Collection and identification of fungi

Field work was conducted at two tree line sites in the Front Range of the Canadian Rockies; between 2,000 and 2,200 m asl on the southeast slope of Mt. Tripoli, in the Nikanassin Range, Alberta (117^0 17'W, 52^0 52'N) and between 2,300 and 2,500 m asl on the southwest slope of Mt. Rae, Peter Lougheed Provincial Park (114^0 59'W, 50^0 36'N). See Chapter Two of this thesis or Kernaghan and Currah in press, for detailed site descriptions.

Sporocarps and ectomycorrhizae were collected along 16 m transects which paralleled

elevational contours through the *Picea/Abies* subalpine forest, the *Salix/Dryas*-dominated alpine zone and the transition zone between the two. Two transects were located approx. 200 m apart in each habitat for a total of six transects at each site. Ectomycorrhizae were collected at 1 m intervals in 1994 by removing 3.5 cm soil cores from the organic soil horizon (down to mineral soil). Methods of handling and identifying ectomycorrhizae are in Kernaghan *et al.* 1997 or Chapter 3 of this thesis.

Sporocarps of all putatively ectomycorrhizal fungi, including epigeous, hypogeous and resupinate forms, were collected within 10 x 26 m quadrats centered around each 16 m transect during each snow-free month (June to Sept.) from 1994 to 1997.

Species richness and diversity indices

Plant species richness values were based on the number of ectotrophic host genera (based on Molina *et al.* 1992) present within the 10 x 26 m quadrats used for sporocarp collection. Richness values for sporocarps and ectomycorrhizae reflect the number of species collected on each transect and were not corrected for sample size. Species diversity of ectomycorrhizae and host plants was quantified by Shannon-Weiner diversity indices.

$$\acute{H} = -\sum_{1}^{S} P_i \ln P_i$$

For ectomycorrhizae, the proportional abundances were the proportion of cores along the transect (0-16) in which a species was recorded. For host plants (*Abies, Picea, Salix* and *Dryas*) the proportional abundances were basal diameter for gymnosperms (measued

with calipers) and visual cover estimates for angiosperms. Diversity indices were not calculated for sporocarp data due to difficulty in quantifying relative abundance.

To determine if there were significant differences in richness and diversity values among the 3 habitats, analyses of variance were performed on data for host plant richness, ectomycorrhizal richness, sporocarp richness, ectomycorrhizal diversity and host plant diversity data. A complete block design was used in which mountains acted as blocks (mountain effects were random and elevation effects were fixed). Because there were no significant mountain effects or mountain x elevation interactions ($p > 0.29$ and $p > 0.18$ respectively for all analyses), data were re-analyzed without the mountain term as a one way ANOVA and Tukey tests were used subsequently to test for significant differences among transects if the results of the initial ANOVA were significant. All analyses were performed using SPSS 7.5. (SPSS Inc., Chicago IL.).

Environmental factors

For determination of abundance of *Abies, Larix* and *Picea*, basal diameter of each stem on each 10 x 26 quadrat was measured and totaled. Stand age was determined by taking increment cores of the two largest trees on each quadrat. For *Salix* and *Dryas*, percent cover on each quadrat was visually estimated. Soil temperature amplitude was determined by burying max./min. thermometers on one transect at each elevation at each site, so that the bulbs were situated in the organic horizon. Thermometers were read each month during the sampling period (and duplicate values were assumed for the replicate transects). Organic material depth (total of L, F and H horizons) was measured at four locations

along each transect and averaged.

For pH and soil moisture determinations, 10 x 10 cm sections of organic horizon were removed adjacent to the ends and sides of each transect each month from June to Sept. 1994. Ten gram subsamples were dried at 60° C for 24 hours and weighed again. Differences between the original and oven dried weights provided moisture content values. A second set of 10 gram subsamples were air dried and then homogenized in a household blender. Two grams of each of these subsamples was mixed with distilled water at 1:10 wt./vol. and left overnight. The samples were then stirred and pH determined with a digital pH meter. Geometric averages of the four pH values were used in subsequent analyses.

Correspondence analyses

Correspondence analysis (CA) was performed on the sporocarp data. Data input was in the form of a species-quadrat data matrix, in which cells contain presence/absence data on the 68 fungal species collected on the 12 quadrats. Canonical correspondence analysis (CCA) (Ter Braak 1987) was also performed on this data set using abundance of the different ectotrophic host plants as the environmental variables to constrain the ordination axes. The abiotic factors (pH, depth of organic material, soil moisture, and temperature amplitude) as well as stand age were highly correlated surrogates of host plant composition and, as such, were added subsequently to the ordination diagram as passive variables. The ordinations of the sporocarp data were detrended (DCA and DCCA) (Hill and Gauch 1980) to alleviate observed arch effects. CA and CCA were also performed on the ectomycorrhiza data. In this case, cells in the species-transect matrix represented

frequency of occurrence (0-16) of 19 distinguishable ectomycorrhizae in soil cores along the transects. All ordination analysis were performed using CANOCO software (Ter Braak 1988).

Results

Sporocarps and ectomycorrhizae collected

A total of 68 ectomycorrhizal taxa were collected as sporocarps within the 12 quadrats; 49 in the subalpine forest, 24 in the ecotone and 11 in the alpine zone (Table 5.1). Fungi belonging to 18 genera were collected in the subalpine forest, a subset of nine of these, plus the genus *Suillus*, was collected in the ecotone, and a further subset of five collected in the alpine zone. *Cortinarius* was by far the most species rich genus in all habitats, although the number of species decreased with elevation. *Hygrophorus, Inocybe* and *Tricholoma* also showed similar trends. The number of *Russula* and *Lactarius* species, however, was greatest at the ecotone (Fig. 5.1). For details on taxonomy, distribution and ecology of the ectomycorrhizal fungi collected, see Chapter Two of this thesis or Kernaghan and Currah in press.

Ectomycorrhizae collected along the 12 transects were sorted among 13 fungal genera and six unidentified fungal taxa. All 13 identified genera, as well as four unidentified taxa, *Cenococcum geophilum* was the most common mycobiont and its abundance varied little among habitats (Fig. 5.2). Mycorrhizae formed by *Cortinarius* were less abundant but exhibited a similar trend. *Amphinema byssoides*, as well as several less abundant genera, decreased in abundance with elevation. The mycorrhizae of *Inocybe, Tomentella* and two

unidentified ascomycetes increased in abundance with elevation and *Russula* and *Lactarius* mycorrhizae were most abundant at the ecotone. For detailed discussion of ectomycorrhizae see Kernaghan *et al.* (1997) and Chapters Four and Five of this thesis.

Species richness and diversity indices

Richness of ectomycorrhizal fungi based on sporocarp collections was significantly different among habitats ($F = 13.863$, $p = 0.002$), with the greatest descrease in mean richness values occuring between the subalpine forest and the ecotone (Fig. 5.3). Variability in sporocarp richness was greater among ecotone transects than among transects in other habitats. Mean richness values for ectomycorrhizae were lower than those for sporocarps, but also decreased from the subalpine forest to the ecotone ($F = 5.792$, $p = 0.024$). The greatest within habitat variability was also at the ecotone (Fig. 5.3).

Richness values based on the number of genera of ectotrophic host plants were highest at the ecotone ($F = 5.727$, $p = 0.025$), while within habitat variability was similar among the three elevations (Fig. 5.3).

Differences in mean Shannon-Weiner diversity indices were similar to differences seen in richness values; ectomycorrhizal diversity decreased in a manner similar to ectomycorrhizal richness ($F = 3.376$, $p = 0.081$) and host plant diversity was highest at the ecotone ($F = 18.945$, $p = 0.001$). Within habitat variability was highest at the ecotone for mycorrhizal diversity, but highest in the alpine zone for host plant genus diversity (Fig. 5.4).

Environmental factors

Major trends in measured environmental factors with increasing elevation include; an overall decrease in the depth of organic material, decreasing stand age, decreases in abundance of *Abies* and *Picea* and overall increases in the abundance of *Salix* and *Dryas*. Differences between the two sites include; higher soil pH and more *Salix* in the alpine zone at Mt Tripoli, greater overall temperature amplitude and more *Dryas* and *Abies* in the alpine zone at Mt. Rae, and the presence of *Larix* at Mt. Rae. (Table 5.2).

Correspondence analyses

In the ordinations of fungal species based on presence of sporocarps, the first two axes of the DCA (not shown) had eigenvalues of λ_1 = .752 and λ_2 =.388 and together explained 27.3% of the total variation. The first two eigenvalues of the DCCA were λ_1 = .707 and λ_2 = .286, and together explained 23.8% of the variation (Fig. 5.5.) The eigenvalues of the DCCA were only slightly lower than those of the DCA, and species-environment correlations for the first two axes were .987 and .905. These results imply that host plant abundances were strong determinants of the variation in ectomycorrhizal fungi.

The first, and most important, ordination axis reflects the elevational gradient in vegetation, with fungal species on the left side of Fig. 5.5 occurring mainly in the mature subalpine conifer forest with its deep organic horizon. Species to the right of Fig. 5.5 on the other hand, occur mainly in the *Salix* and *Dryas* dominated alpine zone, with less organic material and greater temperature amplitude. The majority of species are located towards the middle of the first axis, and therefore most commonly occur in the subalpine

forest and the ecotone. The close proximity of alpine site scores in Fig. 5.5 indicates a distinct alpine community characterized by *Inocybe* spp., *Hebeloma* spp., species assigned to *Tomentella s.l.* and *Salix*-associated *Cortinarius* spp.

The second axis appears to reflect differences between the two sites. Species toward the top of Fig. 5.5 were most often found on Mt. Tripoli, with its more calcareous parent material (Mortimer 1978) giving higher soil pH in the alpine zone, and its southern exposure which results in greater temperature amplitude. Conversely, species toward the bottom of the diagram were most often collected at Mt. Rae, where temperatures are less variable, alpine soils are more acidic and *Abies* and *Larix* are more common. Two other groups of species also appear distinct from the rest: 1) those most commonly found with *Picea,* including *Hysterangium separabile* (Hs), *Hydnellum caeruleum* (Hd), *Inocybe rimosa* (I), *Catathelasma imperiale* (Ca), *Boletopsis subsquamosa* (B), *Cortinarius venetus* (Cl), *Cortinarius* sp. (subgen. *Telamonia*) (Ct) and *Sarcodon* spp. (Sa) and 2) those most commonly found with *Abies* and (or) *Larix*, including *Lactarius luculentus* (L), *Russula torulosa* (R), *Russula silvicola* (R), *Cortinarius evernius* (Ct), *Cortinarius* sp. (subgen. *Telamonia*)(Ct) and *Suillus aeruginascens* (Su).

In the ordinations of fungal genera based on records of ectomycorrhizae along the transects, the first two axes of the CA (not shown) had eigenvalues of λ_1 = .281 and λ_2 = .192 and together explained 45.4 % of the total variation. Eigenvalues for the CCA (Fig. 5.6) were λ_1 = .253 and λ_2 = .174 and explained 41.4 % of the variation. As with the sporocarp ordination, the first two eigenvalues of the CCA were only slightly lower than those of the CA and the species-environment correlations for the first two axes were .970

and .972 respectively, again implying that host plant abundances explained a large proportion of the variation in ectomycorrhizal fungi.

The interpretation of the ordination of data on ectomycorrhizae (Fig. 5.6) is similar to that for Fig. 5.5; the location of genera along the first axis indicates their abundance in either the subalpine forest or the alpine zone, and the location along the second axis indicates the likelihood of occurrence at each site. A distinct alpine community is also apparent, and is characterized by mycorrhizae formed by *Tomentella, Inocybe* and the two alpine ascomycetes. Variation along the second axis was not as great as in the ordination of the sporocarp data (Fig. 5.5), but *Hydnellum* and Forest Basidiomycete 2 form a distinct group unique to the subalpine forest at Mt. Rae and *Tubulicrinis* and *Sarcodon* are unique to Mt. Tripoli. *Abies* and *Picea* associates are not well separated.

Major differences between the CCA of taxa based on collections of ectomycorrhizae and the DCCA of taxa based on sporocarp collections include less variation along both axis for ectomycorrhizae, and less influence of soil pH and proportion of *Abies* (i.e. less influence of site variation).

Discussion

Richness and diversity across the ecotone

In contrast to ectotrophic host plants, which increased in richness and diversity at the ecotone, ectomycorrhizal fungi decreased in richness and diversity from the subalpine forest to the ecotone. This observation was somewhat unexpected, given the obligate

symbioses between the ectomycorrhizal fungi and their ectotrophic hosts, but can be explained on the basis of differences in richness between the subalpine forest and the alpine zone. The edge effect is due to the combination of components from the two adjacent communities at the ecotone. If the adjacent communities differ markedly in species richness, it is expected that their amalgamation at the ecotone would result in an intermediate level of richness, as seen in the ectomycorrhizal fungi.

Possible biotic factors involved in the decrease in richness of ectomycorrhizal fungi across tree line include a decrease in age of conifers with increasing elevation. Mycorrhizal richness and diversity have been shown to increase with stand age up to canopy closure (Last *et al.* 1987, Visser 1995, Rao *et al.* 1997, Helm *et al.* 1996), and the age of the conifers at both sites decreased from approximately 100 years in the subalpine forest to less than 10 years in the alpine zone. The angiosperms above tree line may also support fewer symbioses; sporocarp surveys have shown fewer taxa in broad-leaved forests than adjacent conifer forests (Richardson 1970, Bieri *et al.* 1992). Lastly, alpine plants are smaller and fix less carbon, and therefore have less photosynthates available to support ectomycorrhizal fungi (Newman 1988).

With respect to abiotic factors, important edaphic factors may include pH, temperature, moisture, nutrient content, amount of organic material, and soil structure. Indeed, Danielson and Visser (1989) suggest that "soil specificity" or the ability of fungi to inhabit particular soil types, may play as important a role in fungal distribution as plant specificity. Transpiration rates of sporocarps have also been shown to be highly variable and play an important role in the elevational distribution of macromycetes (Moser 1980) and dwarfism,

presumably a strategy to protect against dessication, is a common trait in alpine sporocarps (Favre 1955). Fungi producing elaborate sporocarps with large surface areas are therefore uncommon in exposed wind-blown areas of the alpine zone. Indeed, the community above tree line in the present study was characterized by fungi that produce either small agaricoid sporocarps, such as *Inocybe*, which do not protrude above the ground cover, and those with resupinate sporocarps, such as *Tomentella*, which fruit beneath stones or prostrate *Salix* branches.

In order to help separate the effects of biotic and abiotic determinants of ectomycorrhizal distribution, the number of fungi which could form symbioses with hosts at all elevations, but do not fruit in the alpine zone, can be examined (used as an indication of the influence of abiotic factors). Of the sixty-eight species collected as sporocarps within the quadrats, 17 do not exhibit specificity for either conifers (*Abies* or *Picea*) or angiosperms (*Salix* or *Dryas*) (Chapter Two of this thesis, Kernaghan and Currah in press). Of these, nine taxa (53%) were collected in the alpine zone, while the remaining eight (47%) of the non-host specific taxa, which could form symbioses with alpine plants, were collected only in the subalpine forest or at the ecotone. Because host specificity is not expected to play a role in the distribution of these taxa, variation in their occurrence along the elevational gradients should be indicative of the influence of climatic and (or) soil characteristics. Of course, these fungi may exist in the alpine zone, but the sporocarps were not observed in the five years of routine sampling.

Soil cores taken at the ecotone which contain both angiosperm and gymnosperm ectomycorrhizae can be used as clear indicators of host plant influence. In this situation,

climate and soils are identical and any differences between the mycobionts of angiosperm and conifer roots are due to plant hosts. Some taxa, *i.e. Amphinema byssoides, Cenococcum geophilum* and *Tomentella* spp. were found on both angiosperm and gymnosperm roots in the same core. Others, however, such as members of the *Russulaceae* and the alpine ascomycetes, were found only on gymnosperm or angiosperm roots, respectively. Such comparisons within individual soil cores clearly indicate a high degree of host specificity in some fungal taxa and little or no specificity in others.

There is evidence then, for strong influences of both biotic and abiotic factors on fungal distributions, with the impact of each varying at the species level. Similarities in eigenvalues and species correlation coefficients between the CAs and CCAs, however, strongly indicate that host plant distribution explains most of the variation in fungal species composition. This is in agreement with the findings of Nantel and Neumann (1992) who found high correlation between ectomycorrhizal fungi and host trees regardless of soil characteristics in a mixed forest in eastern Canada. Moreover, much of the decrease in fungal species richness with elevation can be accounted for by a loss of conifer-specific taxa, again indicating that vegetation is the primary determinant of ectomycorrhizal distribution.

Differences in species composition

The community of ectomycorrhizal fungi in the alpine zone was characterized by generalistic fungi; genera such as *Tomentella*, *Inocybe*, *Cenococcum* and *Amphinema* accounted for a larger proportion of mycorrhizae in the alpine zone than in the subalpine

forest. The high proportion of generalists in the alpine zone appears to be due to the low number of fungi which have evolved specificity to *Dryas* and *Salix*, relative to the number of fungi specific to *Picea* and *Abies* (Trappe 1962, Chapter Two of this thesis, Kernaghan and Currah in press).

The higher levels of host specificity in *Abies* and *Picea* relative to *Salix* and *Dryas* can be related to their role in community succession. The angiosperm host genera predominate at an earlier seral stage than *Picea* and *Abies; Dryas* in particular is a nitrogen fixing pioneer, which has been shown to facilitate succession toward *Salix* and then *Picea* after deglaciation in the Canadian Rockies (Blundon *et al.* 1993).

Last *et al.* (1987) describe a successional pattern for the ectomycorrhizal fungi of *Betula* trees in which seedlings are in symbiosis with a few generalistic fungi, with the diversity and specificity increasing with tree age until canopy closure. An alternate hypothesis is provided by Kropp and Trappe (1982) and Molina *et al.* (1992) for conifers in the Pacific northwest. In their model, trees which establish after disturbance and form relatively pure stands (*eg. Pseudotsuga menziesii*, *Alnus* spp. and *Pinus* spp.) tend to exhibit high levels of host specificity compared to later successional plants which become established in their understorey. The more generalistic understorey species are then able to make use of the existing symbionts of the overstorey plants. At our high elevation sites, however, *Dryas* and *Salix* are pioneers in primary succession after deglaciation, and it is expected that selection pressure towards specificity would be lower for these plants than for later stage conifers, because they would be obliged to form symbioses with whichever mycobionts arrive at the beginning of primary succession.

In general, relatively few angiosperm specific mycorrhizal fungi were found above tree line, although the two alpine ascomycetes and some members of the Cortinariaceae are exceptions. Instead, the community of ectomycorrhizal fungi above tree line is composed largely proportion of fungi that are common to all three elevations.

The positions of montane ecotones are mainly determined by climatic conditions, and as such, are likely to be sensitive indicators of global climate change (Kullman 1996, Hansen-Bristow *et al.* 1988). With increasing temperatures due to elevated CO_2 (the greenhouse effect), conifer establishment in many low alpine areas is increasing (Rochefort and Peterson 1996, Kupfer and Cairns 1996). If angiosperm-specific ectomycorrhizal fungi were to dominate the alpine zone, conifer establishment might be inhibited, but the community of ectomycorrhizal fungi associated with ectotrophic alpine plants in the Front Range of the Canadian Rockies is largely generalistic and therefore lends itself well to conifer recruitment above present tree line.

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Table 5.1. Fungal taxa included in analyses, their abbreviations in the ordination diagram and the habitats in which they were collected. Species of *Cortinarius* are labeled by subgenus; Cl = *Cortinarius* subgen. *Leprocybe*, Cm = C. subgen. *Myxacium*, Cp = C. subgen. *Phlegmacium*, Cs = C. subgen. *Sericeocybe* and Ct = C. subgen. *Telamonia*. For clarity, species of *Hydnum* are grouped with *Hydnellum*, *Camarophyllus* with *Hygrophorus* and *Pseudotomentella* with *Tomentella*. For habitat; F = subalpine Forest, E = Ecotone and A = Alpine zone.

Fungal species	Abbr	Habitat	Fungal species	Abbr	Habitat
Amphinema byssoides	A	F E A	*Hydnellum caeruleum*	Hd	F
Boletopsis subsquamosa	B	F	*Hydnum repandum*	Hd	F
Catathelasma imperiale	Ca	F	*Camarophyllus pratensis*	Hg	F
Cortinarius venetus	Cl	F	*Hygrophorus chrysodon*	Hg	FE
C. zinziberatus	Cl	F	*H. erubescens*	Hg	F
C delibutus	Cm	F	*H. korhonenii*	Hg	F E
C. favrei	Cm	EA	*H. pudorinus*	Hg	F
C. calochrous	Cp	F	*H. pustulatus*	Hg	F
C. crassus	Cp	F	*Hysterangium separabile*	Hs	F
C. fulminoides	Cp	E	*Inocybe dulcamara*	I	FE
C. multiformis	Cp	FE	*I. flocculosa*	I	F
C. percomis	Cp	F	*I. lacera*	I	A
C. sp.	Cp	A	*I. lanuginella*	I	A
C. traganus	Cs	F	*I. rimosa*	I	F
C. sp.	Cs	F	*I. whitei*	I	FE
C. albonigrellus	Ct	EA	*I.* sp.	I	E
C. cf. biformis	Ct	FE	*Lactarius alnicola*	L	F
C. brunneus	Ct	F	*L. caespitosus*	L	FE
C. chrysomallus	Ct	F	*L. deliciosus*	L	FE
C. colus	Ct	F	*L. luculentus*	L	E
C. dilutus	Ct	F	*Russula brevipes*	R	FE
C. evernius	Ct	E	*R. integra*	R	FE
C. galerinoides	Ct	EA	*R. silvicola*	R	EF
C. hinnuleus	Ct	FE	*R. torulosa*	R	E

C. inops	Ct	A	*Sarcodon scabrosus*	Sa	F
C. paragaudis	Ct	F	*Sarcodon* sp.	Sa	F
C. scandens	Ct	F	*Suillus aeruginascens*	Su	E
C. triformis	Ct	FE	*Pseudotomentella tristis*	To	F
C. uraceus	Ct	F	*Tomentella ellisii*	To	AF
C. sp. subgen *Telamonia*	Ct	F	*T. sublilacina*	To	A
C. sp. subgen *Telamonia*	Ct	E	*Tricholoma myomyces*	Tr	F
Dermocybe crocea	D	FE	*T. saponaceum*	Tr	FE
Hebeloma crustuliniforme	He	FA	*T. vaccinum*	Tr	F
H. cf. subfastigiatum	He	FA	*T. virgatum*	Tr	F

Table 5-2. Measured environmental values at each habitat at each site. All values exept for temperature amplitude are averages from two quadrats.

Factor	Forest		Ecotone		Alpine	
	Mt. Rae	Mt. Tripoli	Mt. Rae	Mt. Tripoli	Mt. Rae	Mt. Tripoli
soil pH	5.8	5.8	5.5	5.7	5.6	6.6
% soil moisture	53.7	51.3	42.2	52.3	45.2	51.9
organic horizon (cm)	5.8	6.2	2.8	3.5	1.2	1.7
age of conifers (yrs.)	112	110	65	75	10	5
temperature amplitude (°C)	16	19	15.7	23	15.5	27
basal diam. of *Abies* (cm)	1043	1040	1132	1111	20.1	0.5
basal diam. of *Picea* (cm)	508	902	238	421	11.5	12
basal diam of *Larix* (cm)	24	0	3.5	0	0	0
% cover *Salix*	2.5	0	20	37.5	0.8	80
% cover *Dryas*	0	0	0	0	43.5	7.5

Fig. 5.1. Number of fungal species in each genus at each elevation based on sporocarp collections at two sites in the Front Range of the Canadian Rockies.

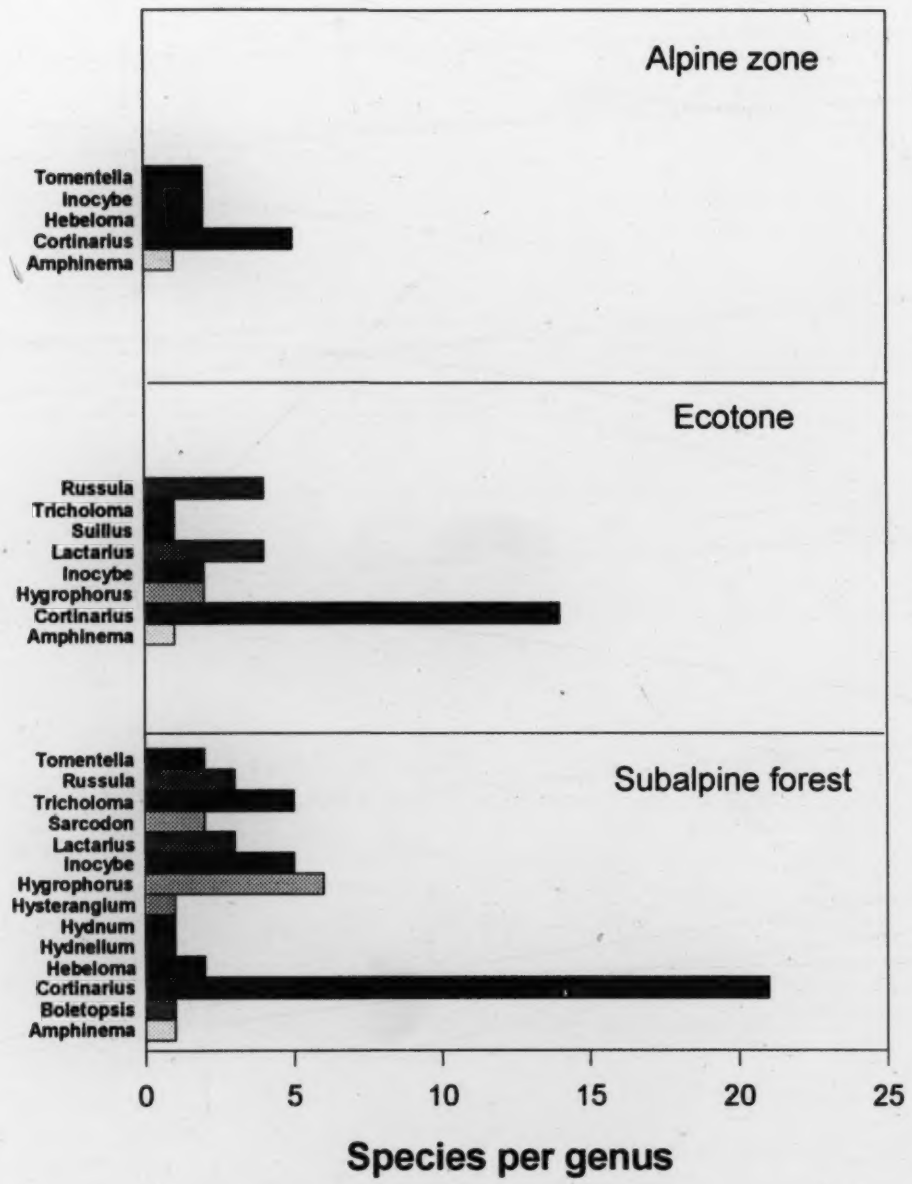

Alpine zone
Tomentella
Inocybe
Hebeloma
Cortinarius
Amphinema
Ecotone
Russula
Tricholoma
Suillus
Lactarius
Inocybe
Hygrophorus
Cortinarius
Amphinema
Subalpine forest
Tomentella
Russula
Tricholoma
Sarcodon
Lactarius
Inocybe
Hygrophorus
Hysterangium
Hydnum
Hydnellum
Hebeloma
Cortinarius
Boletopsis
Amphinema
0
5
10
15
20
25
Species per genus

Fig. 5.2. Number of records of each ectomycorrhiza collected along 16 m transects at each elevation at two sites in the Front Range of the Canadian Rockies.

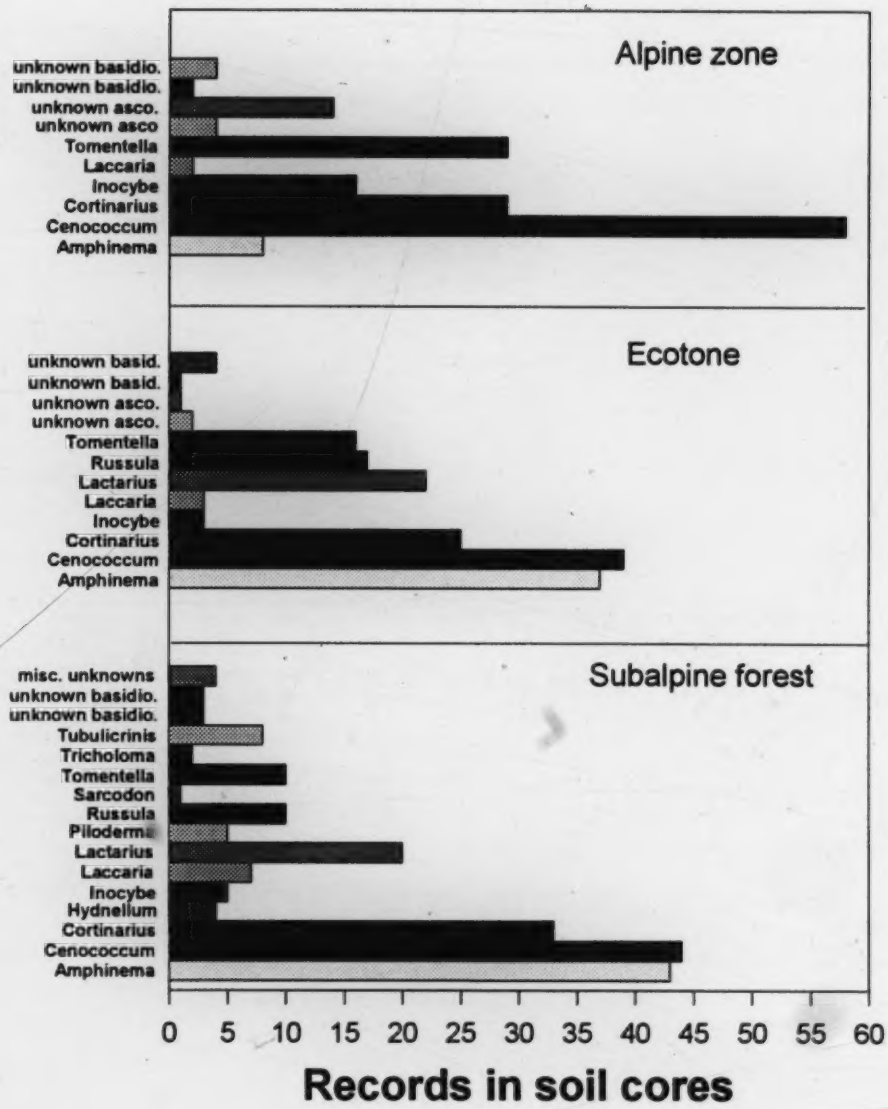
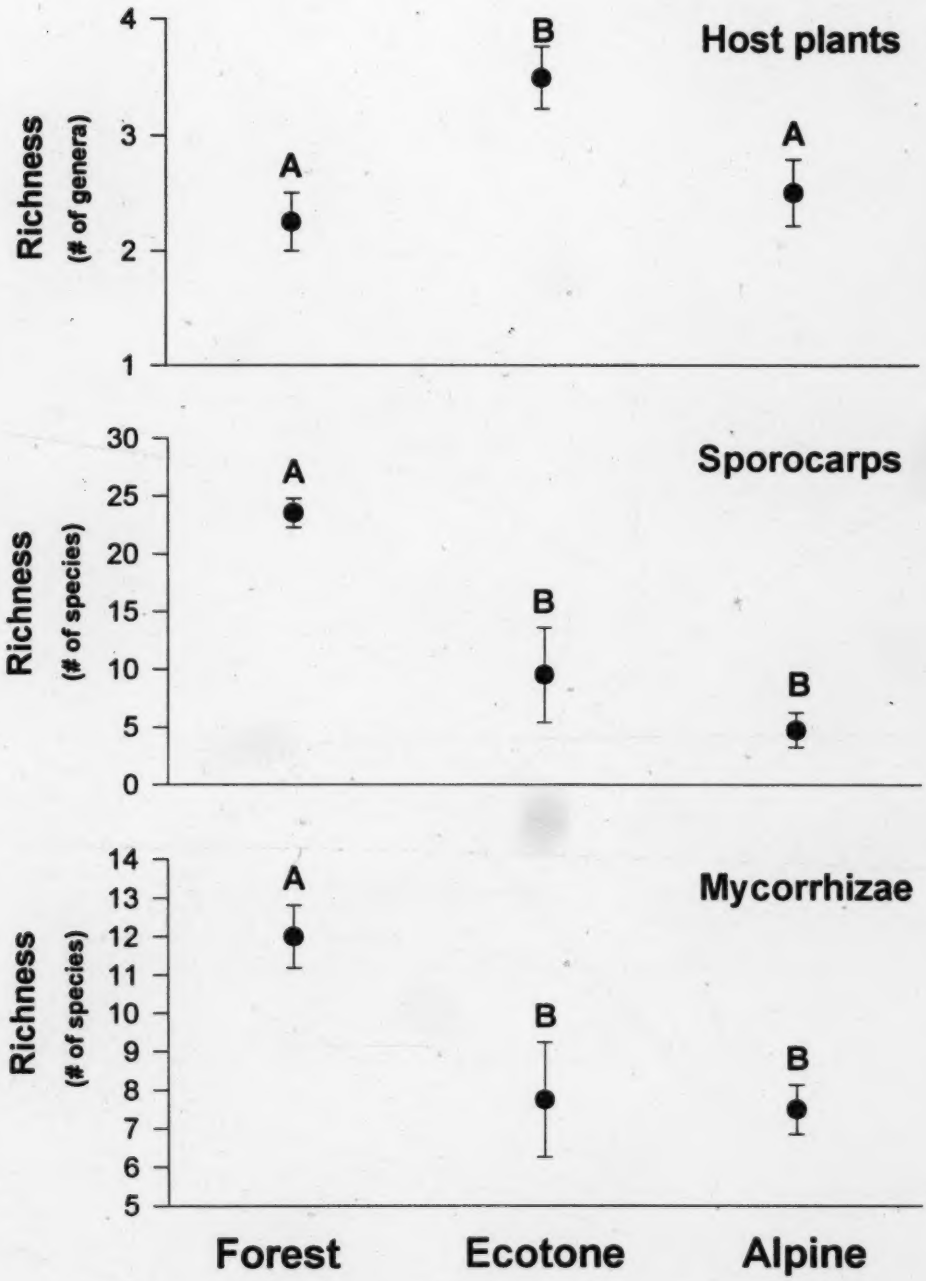

Alpine zone
unknown basidio.
unknown basidio.
unknown asco.
unknown asco
Tomentella
Laccaria
Inocybe
Cortinarius
Cenococcum
Amphinema
Ecotone
unknown basid.
unknown basid.
unknown asco.
unknown asco.
Tomentella
Russula
Lactarius
Laccaria
Inocybe
Cortinarius
Cenococcum
Amphinema
Subalpine forest
misc. unknowns
unknown basidio.
unknown basidio.
Tubulicrinis
Tricholoma
Tomentella
Sarcodon
Russula
Piloderma
Lactarius
Laccaria
Inocybe
Hydnellum
Cortinarius
Cenococcum
Amphinema
0 5 10 15 20 25 30 35 40 45 50 55 60
Records in soil cores

Fig. 5.3. Mean richness (n=4) of host plant genera and richness of ectomycorrhizal fungi based on sporocarp collections and based on ectomycorrhizae. Error bars represent standard error of the mean. Different letters indicate significant differences based on Tukey tests.


Host plants
Richness
(# of genera)
4
3
2
1
A
B
A
Sporocarps
Richness
(# of species)
30
25
20
15
10
5
0
A
B
B
Mycorrhizae
Richness
(# of species)
14
13
12
11
10
9
8
7
6
5
A
B
B
Forest
Ecotone
Alpine

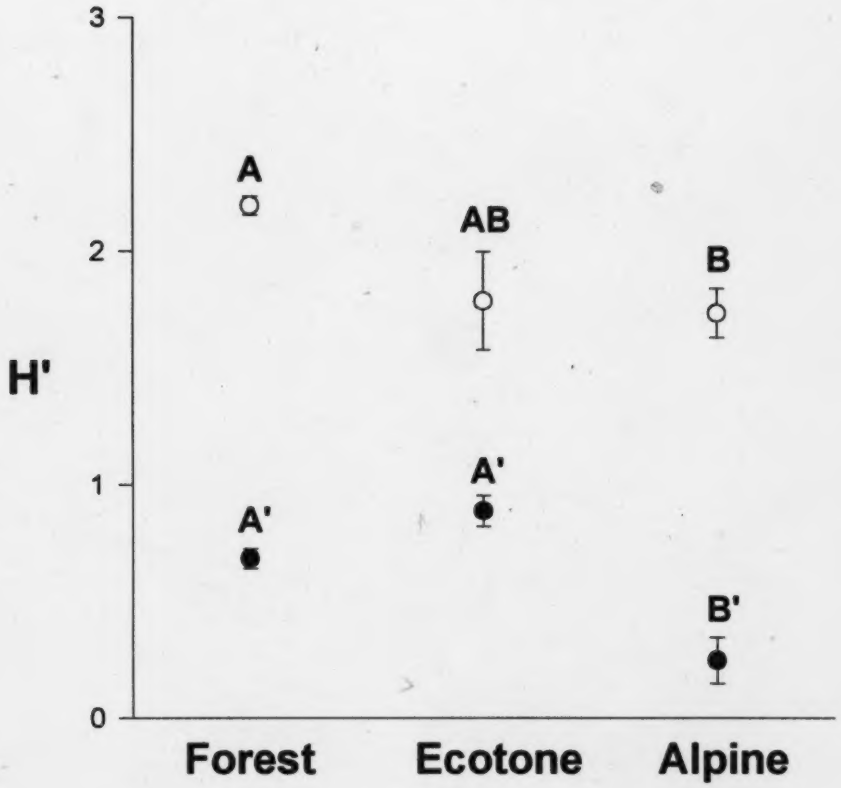


Fig. 5.4. Shannon diversity indices (H') for ectomycorrhizae (hollow circles) and for plant genera (dark circles) at each elevation. Different letters indicate significant differences based on Tukey tests (α = .05 for ectomycorrhizae and 0.1 for host plants).

Fig. 5.5. Detrended canonical correspondence analysis diagram (triplot) of sites, environmental factors and fungal species based on sporocarps. All taxa plotted are labeled with genus or subgenus names for clarity; see Table 5.1 for abbreviations. Sites are labeled as bold capital letters: F=subalpine forest, E= ecotone and A=alpine zone. Environmental factors used to constrain ordination axes are represented by arrows and those added as passive variables are represented by closed circles.

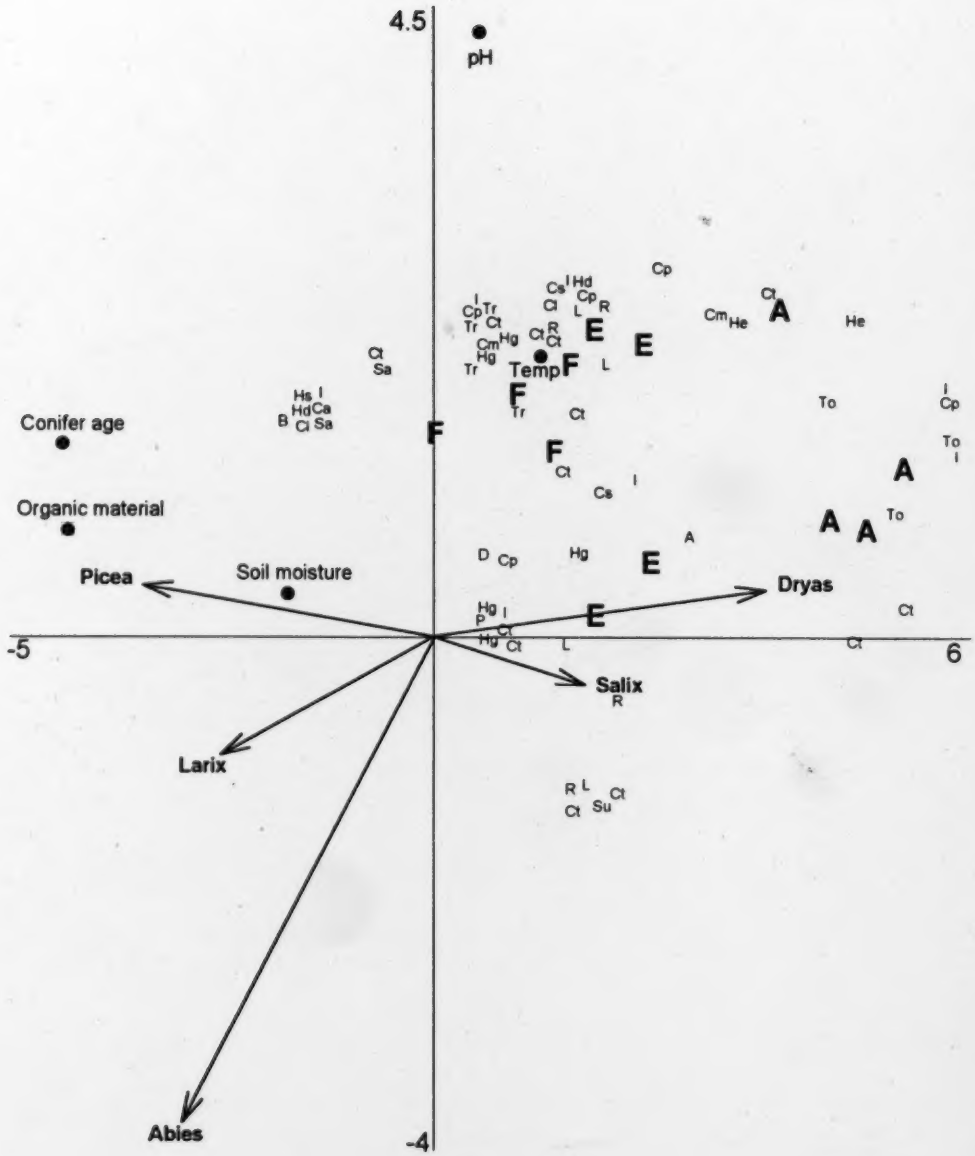
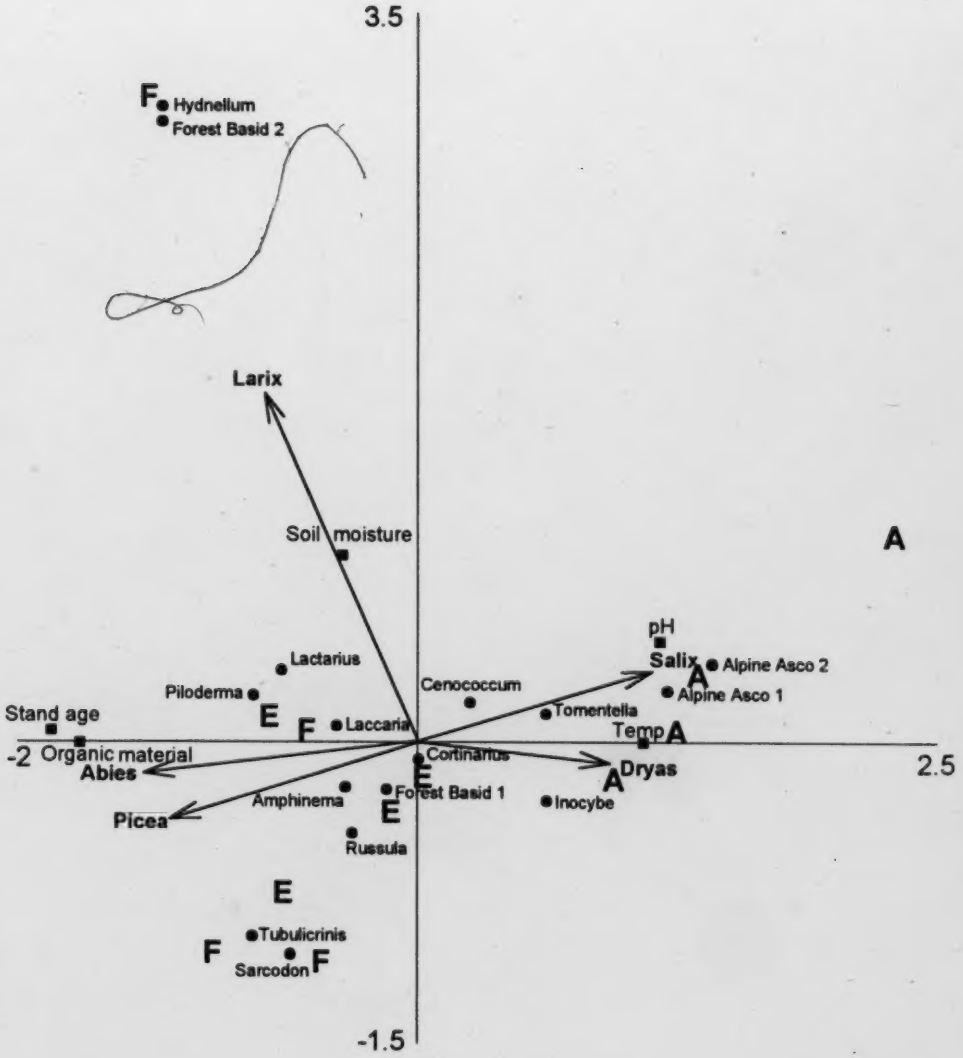

4.5
pH
Conifer age
Organic material
Picea
Soil moisture
Dryas
Salix
Larix
Abies
Temp
-5
6
-4

Fig. 5.6. Canonical correspondence analysis diagram (triplot) of sites, environmental factors and fungal genera based on ectomycorrhizae. Genera are labeled as closed circles. Sites are labeled as bold capital letters. F=subalpine forest, E= ecotone and A=alpine zone. Environmental factors used to constrain ordination axes are represented by arrows and those added as passive variables are represented by squares.


3.5
F
Hydnellum
Forest Basid 2
Larix
Soil moisture
A
pH
Salix
A
Alpine Asco 2
Alpine Asco 1
Lactarius
Piloderma
E
F
Laccaria
Cenococcum
Tomentella
Stand age
Temp
A
-2
Organic material
Abies
Cortinarius
E
Dryas
A
2.5
Amphinema
Forest Basid 1
Inocybe
E
Picea
Russula
E
Tubulicrinis
F
Sarcodon
F
-1.5

Chapter Six

General discussion and conclusions

This research was undertaken in order to help fill two large gaps in our understanding of the ecology of ectomycorrhizal fungi; namely the lack of information regarding the composition of ectomycorrhizal species in western Canada (especially montane regions) and the way in which natural communities of these fungi are structured in different habitats.

The first specific objective was to determine the species composition of ectomycorrhizal fungi on the basis of sporocarp collections on two sites spanning the subalpine alpine ecotone of the Rocky Mountains. A total of ninety five species was documented as sporocarps and (or) ectomycorrhizae, 22 of which are new records for Canada. Species richness based on these sporocarp collections dropped dramatically with elevation, apparently due to host plant composition rather than abiotic factors. Also, two distinct communities were noted; one relatively rich and composed mainly of conifer associates with circumboreal distributions and one relatively poor and composed of species with circumpolar and alpine distributions. At the ecotone, these two communities merged resulting in intermediate levels of species richness.

A total of ninety five ectomycorrhizal fungi is not unexpectedly high when compared to other studies of subalpine *Picea* forests (Bieri 1995, Favre 1960), but the striking difference in richness between the subalpine forest and the alpine zone was surprising. Studies of macrofungi in Swiss alpine *Salix/Dryas* communities by Favre (1955) and Graf (1994) report much higher richness than that of the alpine habitats

studied here.

Although it is difficult to compare results of sporocarp surveys because of differences in scale and sampling strategies, dissimilarities between the Swiss alpine zone and the alpine zone of the eastern slopes of the Canadian Rockies are apparent. The Front Range is located in a rain shadow formed by the Main Range of the Rockies and mean monthly precipitation during the fruiting season (July through September) is between 73 and 110 mm at Mt. Tripoli and between 67 and 77 mm at Mt. Rae, compared to between 90 and 144 at the Swiss sites (Graf 1994). Also, the dominant species of *Salix* encountered during this study (*S. barrattiana*) belongs to section *Lanatae* Koehne which includes species with shrubby growth forms while those encountered in the Swiss studies have truly dwarf growth forms (section *Retusae* Kern.). Sections of *Salix* which include the truly dwarf forms are generally associated with a much larger assemblage of ectomycorrhizal fungi than sections including shrubby forms (B. Senn-Irlet pers.comm.). It would seem that this is an example of a difference, at the host plant section level, in the potential to form symbioses, rather than a difference in host specificity because the *Salix* associates collected during this study are, in general, a subset of those collected in the Swiss studies.

A second objective of this project was to develop PCR-based methods for the identification of ectomycorrhizae for use in combination with traditional anatomical methods. RFLP analysis of ribosomal DNA amplified with fungal specific primers was extemely effective in the identification of the ectomycorrhizae collected during this study. It allowed for the identification and description of 26 species directly from root tips. The

ITS region provided the appropriate level of resolution for the taxa studied, with all species tested giving distinctive RFLP profiles. rDNA analysis is especially well-suited to the identification of mycorrhizae formed by fungi for which *in vitro* resynthesis and hyphal tracing is difficult, and therefore has great potential for the identification and description of ectomycorrhizae formed by fungi with cryptic sporocarps.

Indeed, analysis of ectomycorrhizae revealed a surprising abundance of symbiotic corticioids in all the habitats studied; ectomycorrhizae formed by *Amphinema byssoides* were present in 45% of the soil cores and those of *Tomentella* spp. in 27%. Corticioid fungi are a polyphyletic assemblage grouped together on the basis of fruiting body morphology; prostrate on the underside of decaying wood or rocks or in litter. Although not usually included in surveys of mycorrhizal fungi due to their cryptic nature, other studies on species composition and relative abundance of mycorrhizae also suggest that the corticioid fungi play an important, if not dominant, role. As much as 70% of mycorrhizae in a *Picea sitchensis* forest were formed by *Tylospora fibrillosa* (Taylor and Alexander 1990) and 30% of soil cores in a *Pinus muricata* forest contained *Tomentella sublilacina* (Gardes and Bruns, 1996). Dahlberg *et al.* (1997) also found that the majority of ectomycorrhizae in a *Picea abies* forest were formed by fungi which produce cryptic sporocarps. Species in seven of the 226 corticioid genera in North America have been shown to be mycorrhizal, with many more expected (Ginns and Lefebvre, 1993). The tentative identification of the ectomycorrhizae of *Tubulicrinis* in this study, a genus not previously known to be mycorrhizal, is evidence of the potential importance of symbiotic corticioids in ecosystem functioning.

The success of particular corticioid fungi (*Amphinema* and *Tomentella*) in the subalpine forest as well as the alpine zone may be due in part to an ability of the sporocarps to withstand the desiccating conditions to which more agaricoid sporocarps succumb. The observed fructification beneath stones in the alpine zone would offer protection against high winds and solar radiation, allowing for successful reproduction.

The third and final objective of this study was to compare species composition, richness and diversity of ectomycorrhizal fungi among the subalpine forest, the alpine zone and the intervening transition zone and to relate differences in these aspects to distributional patterns in ectotrophic vegetation.

Both richness and diversity decreased with elevation, in spite of the fact that diversity of ectotrophic host plants was highest at the ecotone, indicating that the diversity of ectomycorrhizal fungi is not necessarily mirrored in that of ectotrophic plants. Canonical correspondence analysis however, indicated that the distribution of ectomycorrhizal fungi across the subalpine/alpine ecotone is determined mainly by the distribution of associated host plants. Host plant distribution is in turn, determined by parent material and climate, which is ultimately governed by topography. The separation of the direct effects of climate and soil on ectomycorrhizal distribution and their indirect effects, via host plant distribution, is a complex problem with many interdependent variables.

The proportion of ectomycorrhizal fungi lacking host specificity increased above tree line, possibly due to the early successional nature of the alpine host plants. This suggests that the alpine ectomycorrhizal community could facilitate conifer establishment

under the appropriate climactic conditions. If the alpine zone was dominated by angiosperm specific fungi however, we might expect the ectomycorrhizal community to play a smaller role in conifer recruitment. The ectomycorrhizal community of the Front Range of the Canadian Rockies may, then, be accelerating the conifer invasion of the low alpine zone seen with increasing temperatures (Rochefort and Peterson 1996).

Research on fungi across ecotones could be expanded by analysis at different spatial scales and (or) at different levels of community organization. Comparable information could be gained by the analysis of ectomycorrhizal fungi across a much larger ecotone, between the boreal forest and the arctic tundra in northern Canada. Alternatively, the complexity could be reduced to the population level and the genetic variation of a single species could be assessed across the ecotone between two stand types.

I feel that this study has contributed significantly to our knowledge of the composition and structure of communities of ectomycorrhizal fungi in montane Canada. As the first analysis of these communities however, it should be viewed as groundwork for future studies. There are undoubtedly many ectomycorrhizal fungi, especially corticioid, left unidentified, and many more questions to be posed.

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APPENDIX 1

Species-quadrat, species-transect and environmental factor data matrices used in ordinations. R = Mt. Rae, T= Mt. Tripoli, F = Forest, E = krummholz zone, A = Alpine zone.

Sporocarp-quadrat matrix

	RF1	RF2	RK1	RK2	RA1	RA2	TF1	TF2	TK1	TK2	TA1	TA2
bolesubs	0	1	0	0	0	0	0	0	0	0	0	0
cortalbo	0	0	0	1	1	1	0	0	0	0	0	0
cortcrass	1	0	0	0	0	0	0	0	0	0	0	0
corttrag	1	0	0	0	0	0	0	0	0	0	0	0
cortcolu	1	0	0	0	0	0	1	0	0	0	0	0
cortbifo	0	1	0	1	0	0	0	0	0	0	0	0
corttrif	1	0	0	1	0	0	1	1	0	0	0	0
cortbrun	1	0	0	0	0	0	1	1	0	0	0	0
cortinop	0	0	0	1	1	1	0	0	1	0	0	1
cortinoc	0	0	0	1	0	0	0	0	0	0	0	0
cortsp1	0	1	0	0	0	0	0	1	0	0	0	0
corturac	1	0	0	0	0	0	1	1	0	0	0	0
cortsp2	1	0	0	1	0	0	0	0	0	0	0	0
cortobtu	0	0	0	0	0	0	0	1	0	0	0	0
cortchry	1	0	0	1	0	0	0	1	0	0	0	0
cortdilu	0	0	0	0	0	0	1	0	0	0	0	0
cortever	0	0	0	1	0	0	0	0	0	0	0	0
cortgala	0	0	0	0	0	0	0	0	0	1	1	0
cortpara	0	0	0	0	0	0	0	1	0	0	0	0
corthinn	0	1	0	1	0	0	0	0	0	0	0	0
cortmult	0	1	0	1	0	0	0	1	0	0	0	0
cortfulm	0	0	0	0	0	0	0	0	0	1	0	0
cortcaloc	1	1	0	0	0	0	0	1	0	0	0	0
cortperco	1	0	0	0	0	0	0	0	0	0	0	0
cortphlg	0	0	0	0	1	0	0	0	0	0	0	0
cortvene	0	1	0	0	0	0	0	0	0	0	0	0
cortzinz	1	0	0	0	0	0	0	1	0	0	0	0
cortdeli	1	1	0	0	0	0	1	1	0	0	0	0
cortfavr	0	0	0	0	0	0	0	0	0	1	1	0
dermcroc	0	1	0	1	0	0	0	1	0	0	0	0
amphbyss	0	0	0	1	0	1	1	1	1	1	0	0
fuscaeru	0	0	0	1	0	0	0	0	0	0	0	0
hebsub	1	0	0	0	1	0	0	0	0	0	1	0

hebcrust	1	0	0	0	0	0	0	0	0	0	1	0
hydnrepa	1	0	0	0	0	0	0	0	0	0	0	0
sarccalv	0	1	0	0	0	0	0	1	0	0	0	0
hydncaer	0	1	0	0	0	0	0	0	0	0	0	0
hystsepa	0	1	0	0	0	0	0	0	0	0	0	0
inocrim	0	1	0	0	0	0	0	0	0	0	0	0
inocdulc	0	0	1	0	0	0	1	1	0	1	0	0
inoclace	0	0	0	0	1	0	0	0	0	0	0	0
inoclanu	0	0	0	0	1	1	0	0	0	0	0	0
inocwhit	0	0	0	0	0	0	1	0	1	1	0	0
inocfloc	0	0	0	0	0	0	1	0	0	0	0	0
inocleut	0	0	0	0	0	0	0	1	0	0	0	0
hygrchry	0	1	0	1	0	0	1	0	0	0	0	0
hygrkorh	1	0	0	1	0	0	1	0	0	0	0	0
hygrerub	0	0	0	0	0	0	1	0	0	0	0	0
hygrpudo	0	0	0	0	0	0	0	1	0	0	0	0
hypustu	1	1	0	0	0	0	1	0	0	0	0	0
camprae	1	0	0	0	0	0	1	1	0	0	0	0
cataimp	0	1	0	0	0	0	0	0	0	0	0	0
trichsapo	1	1	0	1	0	0	0	1	0	0	0	0
tricmyom	1	1	0	0	0	0	1	1	0	0	0	0
tricvacc	1	1	0	0	0	0	0	1	0	0	0	0
tricvirg	1	1	0	0	0	0	0	1	0	0	0	0
lactalni	0	0	0	0	0	0	1	1	0	1	0	0
lactcaes	0	1	0	1	0	0	1	1	1	0	0	0
lactdeli	1	1	1	1	0	0	0	0	1	0	0	0
llucu	0	0	0	1	0	0	0	0	0	0	0	0
russbrev	0	0	0	0	0	0	1	0	0	1	0	0
russinte	0	0	0	0	0	0	1	1	0	1	0	0
russtoru	0	0	0	1	0	0	0	0	0	0	0	0
russsilv	0	0	0	1	0	0	0	1	1	0	0	0
tomsubl	0	0	0	0	1	1	0	0	0	0	0	0
tomelis	0	0	0	0	1	1	0	1	0	0	0	0
pseutom	0	0	0	0	0	0	1	0	0	0	0	0
sarcosca	0	1	0	0	0	0	0	0	0	0	0	0

Transect-mycorrhizae matrix

	RF1	RF2	RK1	RK2	RA1	RA2	TF1	TF2	TK1	TK2	TA1	TA2
amph	8	5	10	10	3	2	14	16	8	9	3	0
cenococc	15	12	4	13	13	14	10	7	13	9	16	15
lactarius	6	7	0	13	0	0	3	3	7	2	0	0
russula	2	0	0	2	0	0	4	5	8	7	0	0
tomentel	3	2	0	2	4	7	2	3	5	9	9	9
cortinar	10	4	5	14	10	10	7	12	6	0	6	3
laccaria	0	2	0	0	0	2	5	0	0	3	0	0
tubulicr	0	0	0	0	0	0	6	2	0	0	0	0
inocybe	0	0	0	0	5	9	0	5	3	0	2	0
alpasco1	0	0	0	0	0	0	0	0	2	0	4	0
alpasco2	0	0	0	0	5	0	0	0	1	0	0	9
sarcodon	0	0	0	0	0	0	0	1	0	0	0	0
piloderm	4	1	0	0	0	0	0	0	0	0	0	0
forbasid1	3	0	0	1	2	0	0	0	0	0	0	0
hydnellu	0	4	0	0	0	0	0	0	0	0	0	0
forbasid2	0	2	0	0	0	0	0	0	0	0	0	0

Quadrat-environmental factor matrix

	RF1	RF2	RK1	RK2	RA1	RA2	TF1	TF2	TK1	TK2	TA1	TA2
averagph	5.9	5.8	5.8	5.2	5.7	5.5	5.8	5.8	5.8	5.6	6.5	6.7
avemoist	48.6	58.8	37.4	47.1	48.1	42.4	57.6	45.0	49.8	54.9	52.1	51.7
standage	100.0	125.0	80.0	50.0	0.0	15.0	115.0	105.0	45.0	65.0	5.0	0.0
tempampl	16.0	16.0	15.8	15.8	15.5	15.5	19.0	19.0	23.0	23.0	27.0	27.0
organicd	5.5	6.2	2.0	3.6	1.0	1.5	6.5	6.0	4.0	3.0	2.0	1.5
larixmas	0.0	48.0	0.0	7.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
abiesmas	1111.0	976.0	482.0	1783.0	1.5	50.0	1253.5	828.0	1528.0	694.0	0.5	0.0
piceamas	361.0	656.0	223.0	253.0	4.0	19.0	817.0	988.0	655.0	187.0	24.0	0.0
perdryas	0.0	0.0	0.0	0.0	40.0	47.0	0.0	0.0	0.0	0.0	15.0	0.0
persalix	5.0	0.0	20.0	20.0	5.0	0.5	0.0	0.0	50.0	25.0	70.0	90.0

APPENDIX 2

Fungi collected as sporocarps (S) and (or) mycorrhizae (M) on both sites.

Fungi collected	Tissue	Fungi collected	Tissue
Albatrellus flettii	S	*Hydnotrya cubispora*	S
"Alpine Ascomycete" 1	M	*Hydnum repandum*	S
"Alpine Ascomycete" 2	M	*Hygrophorus chrysodon*	S
Amanita vaginata	S	*H. erubescens*	S
Amphinema byssoides	S M	*H. korhonenii*	S
Boletopsis subsquamosa	S	*H. speciosus*	S
Camarophyllus pratensis	S	*H. pustulatus*	S
Catathelasma imperiale	S	*H. pudorinus*	S
Cenococcum geophilum	S M	*Hysterangium separabile*	S
C. albonigrellus	S	*Inocybe dulcamara*	S
C. brunneus	S	*I. flocculosa*	S
C. calochrous	S M	*I. lacera*	S
C. chrysomallus	S	*I. lanuginella*	S
C. clandestinus	S	*I. rimosa*	S
C. colus	S	*I. whitei*	S
C. crassus	S	"Alpine Inocybe"	M
C. delibutus	S	*Laccaria montana*	S
C. dilutus	S	*Laccaria* sp.	M
C. evernius	S	*Lactarius alnicola*	S M
C. favrei	S	*L. caespitosus*	S M
C. fulminoides	S	*L. deliciosus*	S M
C. galerinoides	S	*L. luculentus*	S
C. glaucopus	S	*L. pubescens*	S
C. himnuleus	S	*Piloderma byssinum*	M
C. inops	S	*Pseudotomentella tristis*	S
C. multiformis	S	*Russula brevipes*	S M
C. muscigenus	S	*R. integra*	S M
C. orichalceus	S	*R. silvicola*	S M
C. paragaudis	S	*R. torulosa*	S
C. percomis	S	*Rhizopogon rubescens*	S
C. scandens	S	*Sarcodon scabrosus*	S
C. tragamus	S	*Sarcodon cf. versipellis*	S
C. triformis	S	*Sarcodon* sp.	M
C. uraceus	S	*Suillus aeruginascens*	S
C. venetus	S	*Suillus cavipes*	S

C. zinziberatus	S	*Tricholoma myomyces*	S
"Alpine Cortinarius"	M	*T. saponaceum*	S
"Forest Cortinarius" 1	M	*T. vaccinum*	S
"Forest Cortinarius" 2	M	*T. virgatum*	S
"Krummholz Cortinarius"	M	*Thaxterogaster pingue*	S
Dermocybe crocea	S	*Thelephora caryophyllea*	S
Gomphidius largus	S	Thelephoraceae 1	M
Hebeloma crustuliniforme	S	Thelephoraceae 2	M
Hebeloma insigne	S	Thelephoraceae 3	M
H. cf. subfastigiatum	S	*Tomentella ellisii*	S
Hydnellum caeruleum	S M	*Tomentella sublilacina*	S
Hydnellum suaveolens	S	*Tubulicrinis* sp.	M